Intelligent Implants to Battle Biofilms

Self-diagnosing, self-treating, self-monitoring artificial joints could combat postimplant infections attributable to biofilms

Garth D. Ehrlich, Fen Ze Hu, Qiao Lin, J. William Costerton, and J. Christopher Post

"The extreme morbidity associated with infected prosthetic joints demands attention"
—Nicholas Sotereanos, Orthopedic Surgeon

We plan to develop a self-diagnosing, self-treating, self-monitoring artificial joint—an intelligent implant—to combat postimplant infections that form when microbial biofilms form on prostheses (Fig. 1).

According to both surgeons and clinical microbiologists, more than 90% of infections on artificial joints result from two related staphylococcal species: *Staphylococcus aureus* and *S. epidermidis*. Microbiologists agree that such infections need to be controlled before biofilms form because available regimens do not eradicate biofilms on abiotic surfaces implanted in human tissues. Microelectromechanical systems (MEMS) engineers say that the most difficult technical challenge is to overcome biofouling of implanted devices that contain membranes through which ligands pass. Electrical engineers say that powering such devices for years without external leads, which would be a source of infections, is another formidable challenge.

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These consensus viewpoints are helping us to design an intelligent implant.

The Clinical Challenge

Few medical problems are more devastating to patients and physicians than an infected artificial joint. Removing an infected prosthesis is traumatic, as it entails shattering surrounding bone and removing nearby soft tissues. Although patients are under anesthesia during this harrowing procedure, they awake to find their affected limbs attached to the body only by damaged soft tissues. This condition typically confines such patients to bed rest for months, during which they receive intravenous (IV) antibiotic therapy and must remain immobile.

Following IV therapy, such patients face an additional round of replacement implant surgery, often involving much larger implants depending on how much bone was destroyed during previous implantation and excision procedures. Moreover, reimplants typically have higher rates of infection than do primary joint replacements. For instance, secondary total hip replacements have a 3.2% reinfection rate, while secondary total knee replacements have a 5.6% reinfection rate. Even more troubling, failure of revisional surgery typically leads to amputation.

Arthroplasties are not rare, with U.S. hip or knee replacements each surpassing 200,000 annually. Moreover, these numbers are expected to increase during the next decades because of an aging population with added risk factors, including high-impact exercise for many and obesity with concomitant endemic osteoporosis for many others. Estimated overall rates of infection for artificial joints, including for primary and reimplants, fall within the 1–2% range, suggesting as many as 8,000 infected arthroplasties annually.

Revisional surgery and care now cost in excess of $500,000 per patient, amounting to more than $2 billion annually. Such costs easily

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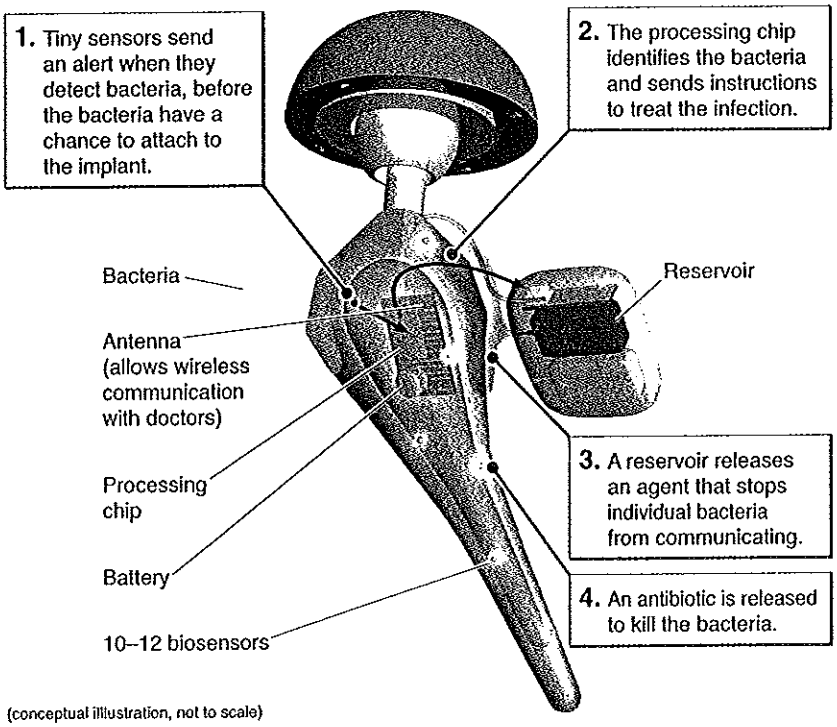
FIGURE 1



Schematic diagram of an artificial hip joint showing the major components associated with the development of an intelligent implant which has the capability to diagnose and treat nascent bacterial infections, as well as provide feedback to the patient and physician via telemetry. Graphics developed by Cindy Jones-Hulfachor in consultation with the author, reproduced with permission from the *Sun-Sentinel*.

justify a greater investment toward developing and manufacturing intelligent implants. Such devices would be engineered to have multifunctional, self-actuated systems that would include bacterial diagnostics, automonitoring treatment regimens, and telemetry to provide feedback regarding the microbiological and pharmacological state of the joint.

Toward this end, we are assembling a multidisciplinary team of engineers, surgeons, and scientists who will identify problems and develop practical plans for combating them. We have established an executive council to direct and coordinate these team efforts, with the long-term goal of creating an artificial joint to reduce infection risks by prophylaxis and thereby minimize the need for revisional surgeries.

To achieve this goal, we will also need to partner with implant manufacturers, a step that is fraught with legal difficulties. For instance, marketing an improved medical device could be construed as an admission that earlier versions were defective, perhaps encouraging lawsuits. Federal legislation may be needed to protect manufacturers from such liability lawsuits if we are to move forward with building improved prostheses.

Biofilms Define the Microbiological Problem

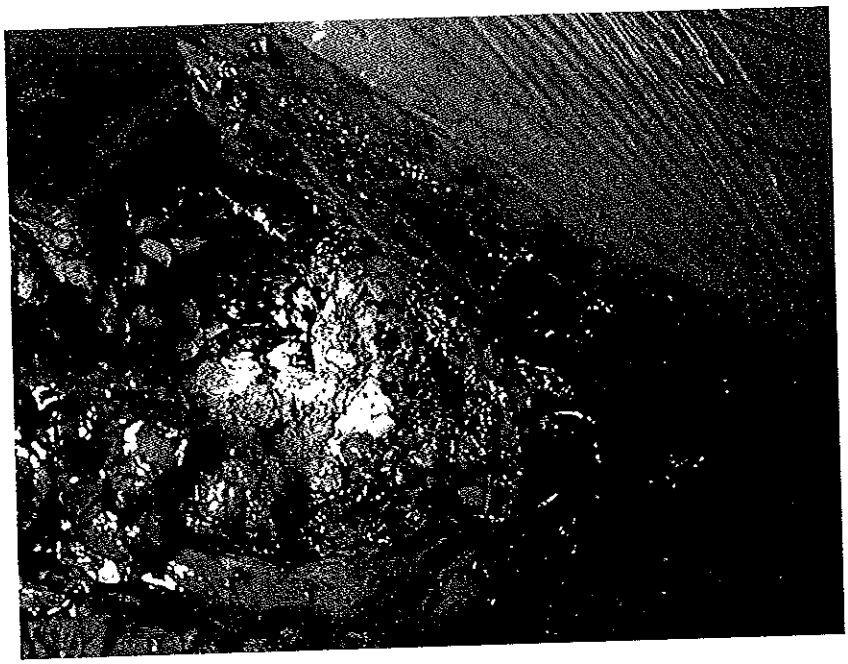
Like other devices, arthroplasties are prone to supporting biofilms, including separate or mixed infections of bacterial and fungal species (Fig. 2). Biofilms are not simply collections of individual bacteria. Instead, they are complex cooperative communities of microorganisms that contain one or more species embedded within an extracellular matrix—displaying discrete temporal and spatial organization, and possessing environmental sensing mechanisms whose adaptive responses operate at the population level.

Biofilm bacteria can become a permanent feature of an infected device, meaning there may be no means—short of removing it or killing the host—to eradicate the biofilm. Such biofilm bacteria thus are nearly impervious to host defense mechanisms or intensive long-term antimicrobial therapy. We suspect that these resistances result from bacterial biofilms functioning in part as single living units, analogous to metazoans. Because bacteria in biofilms face selection pressures at the population level and as individuals, this duality provides them with extraordinary fitness.

With abundant nutrient sources and absent severe environmental threats, bacteria will grow as planktonic, or free-living, blooms. However, faced with adversity, they may attach to surfaces and form biofilms. Traditional approaches, such as isolating strains to study in pure culture, have proved inadequate for understanding chronic infections that are recalcitrant to ordinary therapies and host defenses.

Thus, we developed the concept of bacterial plurality that embodies the extensive pheno-

FIGURE 2



Intraoperative photograph taken during excisional surgery to remove an infected prosthetic joint demonstrating a mixed-kingdom biofilm composed of bacterial and mycal species on an infected arthroplasty. The white material is pus that contains huge numbers of microorganisms and host-derived leukocytes.

·ypic and genotypic diversity that can ·ccur within a biofilm. Bacteria can persist within a biofilm partly because they are situated within so many different microenvironments—each varying because of differences in conditions such as nutrient availability, pH, and oxidizing potential. In adapting to these niches, bacteria within a biofilm display numerous phenotypes, with broad metabolic and replicative heterogeneity, providing the community as a whole with enormous capacity to withstand challenges, whether from host defense systems or pharmaceuticals.

For instance, a biofilm may exhibit antibiotic resistance levels that are three or more orders of magnitude greater than those displayed by planktonic bacteria of the same strain. When antibiotic treatments to biofilms result in multilog kills, the number of truly resistant organisms within a biofilm typically is small. However, the minority of drug-resistant bacteria that persists after treatment tends to repopulate the ·ofilm. Furthermore, subsequent treatments of the repopulated biofilm only modestly reduce bacterial numbers, indicating that the repopulated biofilm is more resistant to the treatment. However, once dispersed from the repopulated biofilm, planktonic bacteria typically remain antibiotic sensitive, indicating that resistance is part of an inducible process among bacteria within the biofilm.

From a clinical perspective, these changes mean that traditional antibiotic therapy will never be successful against biofilm bacteria and that other modes of treatment will be needed to prevent biofilm formation in the first place. Even a 5- or 6-log kill is useless when there is a nidus of infection that persists and which will immediately repopulate the biofilm with mostly resistant bacteria.

Genotypic Diversity within Biofilms Complicates the Challenge

Bacterial plurality within biofilms embodies genotypic diversity that can be thought of in terms of two separate phenomena. The first, genetic ·erogeneity, is defined as different organisms ·ithin a population or species having different alleles of the same set of genes—a widely recognized feature of natural populations.

The second phenomenon, genomic plasticity, is defined in terms of individual strains within a population that each have a unique distribution of contingency genes (not alleles) from a population-based supragenome. According to this concept, also termed the distributed-genome hypothesis, all organisms of the same species do not have the same set of genes, and no organism contains the full complement of genes of the species. A corollary to the distributed-genome hypothesis is that the supragenome is far larger than the genome of any single bacterial species within a population.

Ongoing studies by scientists at the Center for Genomic Sciences at Allegheny Singer Research Institute in Pittsburgh, Pa., are providing tantalizing clues that infections often are polyclonal. The presence of multiple strains, each with a unique genomic complement, provides for reassortment of genes among strains and could be a general mechanism enabling infections to persist.

Several threads of circumstantial evidence as well as direct comparative sequencing studies

support the distributed-genome hypothesis. The first line of circumstantial evidence is the evolution and maintenance of the autocompetence and autotransformation systems for the uptake of foreign DNA by both gram-negative and gram-positive pathogens. Although the overall genome size of many of these pathogens is very small, the number of genes and operons involved in encoding these functions is very large.

The retention of these genes in the face of enormous selective pressure to minimize genome size strongly indicates selective pressure for these bacteria to maintain their capacity to take up DNA from the environment. The fact that some of these organisms may use nucleic acids as a nutrient source is insufficient to explain why only one strand is degraded upon uptake, with the second strand remaining available for transformation; why some species have developed species-specific signal uptake sequences that preferentially ensure the DNA brought into the cell is from their own species; and why there are separate enzymatic functions encoded within the various competence operons that are not required for nucleic acid catabolism but are absolutely required for DNA integration and transformation.

The second provocative line of circumstantial evidence is that the principal component of the extracellular matrix within the *Pseudomonas aeruginosa* biofilm is DNA—in other words, these bacteria are essentially taking a bath in DNA. Moreover, according to studies at the Center for Genomic Sciences, Pittsburgh, Pa., 10–15% of genes in recent clinical isolates of *Haemophilus influenzae, Streptococcus pneumoniae,* and *P. aeruginosa* are unique when compared with sequences of laboratory type strains.

Evolutionary origin studies, however, indicate that these seemingly novel genes existed within the respective species' supragenome for as long as most of the other genes within that genome. Moreover, distribution studies demonstrate that no two of these clinical strains within a particular species have the same set of these seemingly novel genes. The extraordinary genomic plasticity among these clinical strains implies that each receives unique genes from the population supragenome. This observed genomic plasticity, along with ready mechanisms for continuously creating novel gene combinations through reassortment, further support the distributed genome hypothesis.

Meanwhile, other studies of DNA exchange in vitro indicate 10-fold-higher rates of transformation in biofilm bacteria than among planktonic forms. Thus, bacteria in biofilms apparently reassort genes continuously, thereby producing huge numbers of novel strains, some small percentage of which will have a selective advantage for the particular host environment in which they find themselves. This genomic dynamism thus helps to explain why biofilm infections persist.

Moreover, these observations partly explain why it is so difficult to develop chronic bacterial infections in animal models. Thus, when researchers deliberately infect animals with single clonal isolates, the pathogens simply do not have sufficient genomic diversity for the reassortments and adaptations needed for them to persist in otherwise healthy animals with intact immune systems.

An Engineering Approach To Meet These Challenges

The concept behind our intelligent implant is to design an arthroplasty with a MEMS-type biosensing device that, figuratively, eavesdrops on bacterial communication systems associated with autoinduction, quorum sensing, and biofilm formation. Both pathogenic and nonpathogenic bacterial species produce and respond to intercellular signaling molecules that either sense the concentration of bacteria in a given locale (quorum sensing) or determine rates of diffusion within the ecosystem in which the bacteria find themselves.

Depending on bacterial species and environment, quorum sensing coordinates metabolic switching among members of a population of like bacteria, enabling them to act in concert and to benefit the population instead of individual organisms. For many pathogens, detecting a quorum of bacteria induces production of virulence factors and toxins. This coordinated phenomenon at the population level appears to be a survival strategy for pathogens, keeping them below the radar of a host's pathogen-detection systems until their numbers are high enough to overwhelm that host's initial defenses. An example of this phenomenon is the *S. aureus*-induced toxic shock syndrome.

Our MEMS biosensor will respond to bacterial communication signals through integral, gated reservoirs that will release compounds to

...nt biofilm formation along with high concentrations of antibiotics to eradicate nearby planktonic bacteria. With site-specific dosing, the intelligent device could administer far higher concentrations of antibiotics locally than could be tolerated by the host through parenteral or other systemic treatment regimens and might also permit use of antimicrobials with specific organ system toxicity profiles. The MEMS sensors and reservoirs also would be connected to a telemetry system embedded within the prostheses that could enable physicians to monitor patients anywhere in the world.

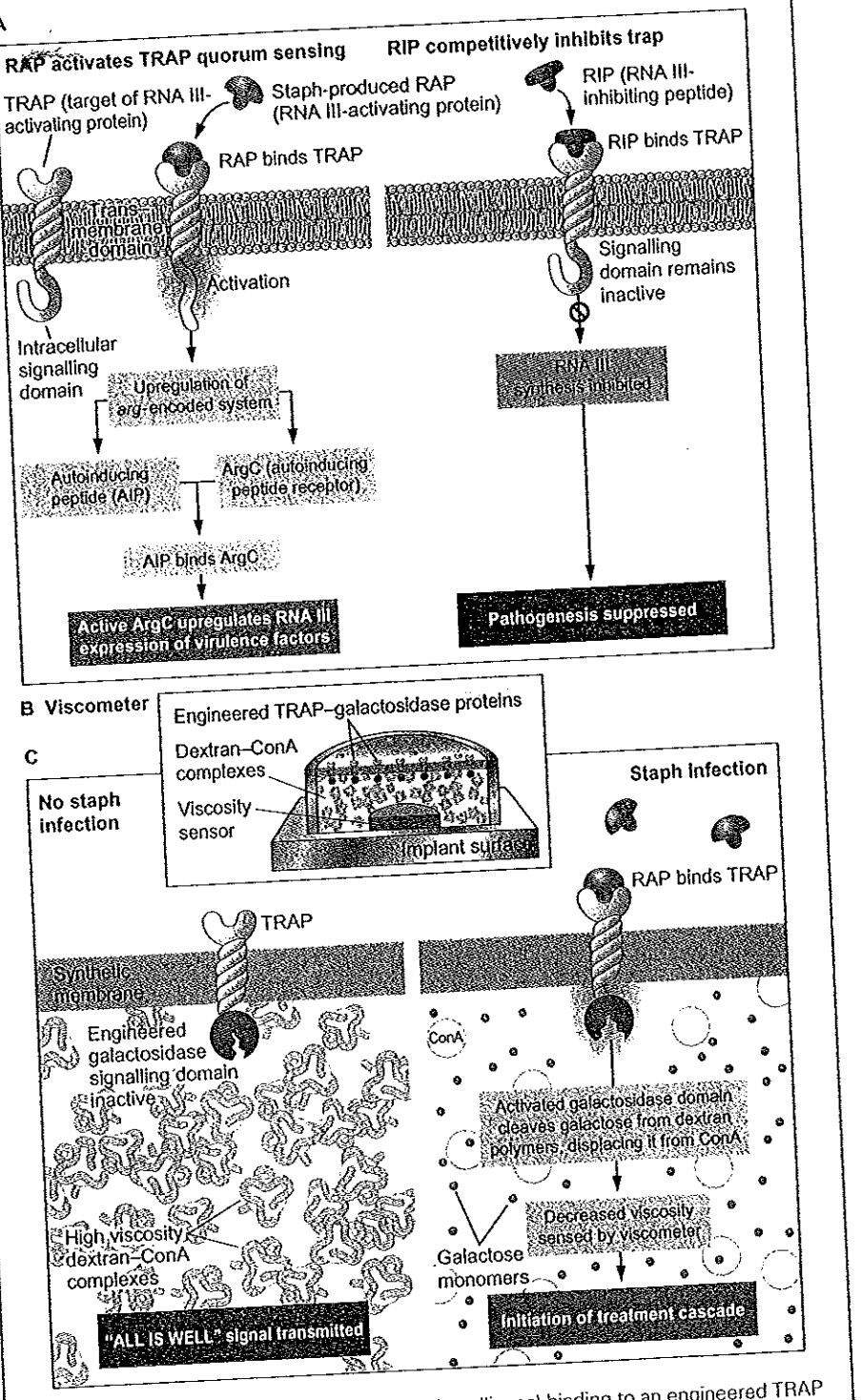
Several Steps for Developing an Intelligent Implant Appear within Reach

The predominant bacterial pathogens associated with artificial joints, *S. aureus* and *S. epidermidis*, account for more than 90% of all implant infections. Because both these staphylococci use the same peptide-based quorum sensing system, we are focusing exclusively on these organisms while developing a first-generation intelligent implant.

Specifically, both species produce a quorum-sensing peptidyl autoinducer ligand, called RAP (RNA III-activating protein), and a cognate cell-surface based receptor, called TRAP (target of RNA III-activating protein), which is phosphorylated when it binds RAP. Activating TRAP upregulates a second two-component system encoded by *agr*. Activating this secondary signaling system, in turn, produces autoinducing peptide (AIP) and AgrC, its cognate receptor. When AIP binds to AgrC, the resulting phosphorylation cascade upregulates synthesis of RNA III, which controls expression of numerous virulence factors.

The *agr* locus contains several different transcriptional systems that are controlled by the P2 and P3 promoters, which encode RNA II and RNA III, respectively. Thus, P2 is activated by the ...P-TRAP system, and P3 by the AIP-AgrC system.

FIGURE 3



(A) Schematic of bacterially produced RAP (blue ellipses) binding to an engineered TRAP that contains an intraluminal galactosidase that is activated upon RAP binding from an inactive form (brown) to an active form (green). (B) Schematic of a MEMS cantilever-based biosensor which can detect viscosity changes associated with galactoside activity triggered by RAP binding. (C) Operation of the biosensor under uninfected and infected conditions.

We are designing the first MEMS biosensor to detect the earliest stage of staphylococcal intercellular communication, thus providing the maximum window for treatment. Our goal is to create a biosensor based on TRAP that would bind bacterially produced RAP (Fig. 3). Already we know that interfering with the RAP-TRAP signaling system by the peptide RIP (RNA III inhibiting peptide) can be beneficial by reducing staphylococcal-based pathogenesis, according to Naomi Balaban of Tufts University in Boston, Mass. RIP, an octapeptide, is produced by some coagulase-negative staphylococci, including *S. warnerii* and *S. xylosus*, and competitively inhibits binding of RAP to TRAP, making RIP a highly effective inhibitor of RNA III synthesis in vitro and suppressing pathogenesis in vivo. Moreover, when mice are vaccinated with RAP, they are protected when challenged with *S. aureus*.

The binding of RAP to the biosensor would initiate a signal transduction cascade within the MEMS device that would release RIP and anti-RAP antibodies from the implant's reservoirs. They would flood the space near the prosthesis, preventing toxin production, biofilm development, and quorum sensing by planktonic staphylococci. Simultaneously, a second set of reservoirs would release antibiotics, such as methicillin and perhaps vancomycin, to kill planktonic staphylococci. In turn, a second set of MEMS-based sensors would monitor to ensure that these releases are adequate. Meanwhile, a record of these activities would be stored in a module embedded in the implant that patients receive, and those data could readily be transmitted to their physicians.

Developing and integrating the MEMS biosensors loom as critical steps for the entire intelligent implant system. On the positive side, the biology for controlling staphylococcal infections is well worked out, while the controlled-release reservoirs most likely will depend on modifying off-the-shelf valve systems, gating components, and biocompatible reservoir materials. Even suitable telemetry systems are available, albeit in different guises.

Hurdles Include Developing a Generic Biosensing Core

For simplicity and cost control, we plan to develop a MEMS device with a cantilever-based viscometer as its sensor. This device is based on the approach adopted by Joel Jeckelmann and A. Seibold of Disetronic Medical Systems AG, Burgdorf, Switzerland and Sulzbach, Germany, to monitor blood glucose levels. Their device measures changes in viscosity based on competition between free glucose and dextran polymers in binding the lectin concanavalin A (ConA). Each ConA molecule can bind four dextran molecules, yielding a high-viscosity matrix. Free glucose competes for those binding sites, lowering viscosity.

Our goal is to adapt this approach to a system in which RAP binding will release glucose from a polymer. This strategy should circumvent the problem of biofouling because we will not attempt to pass the ligand to be detected (RAP) into the biosensor. Instead, we will transduce a signal into the biosensor using a transmembrane-based conformational protein switch. Specifically, we plan to take a protein-engineering approach, fusing the extracellular domain of TRAP to a transmembrane conformational switching domain that, in turn, is fused to a galactosidase.

When the hybrid TRAP molecule is unbound, the galactosidase inside the biosensor will be in an inactive conformation and the biosensor will transmit a high-viscosity, all-is-well signal. If RAP produced by staphylococci is present near the joint, it will bind to the engineered TRAP proteins and the conformational shift will activate the galactosidase, releasing glucose from a polymer, displacing dextran from ConA, and decreasing viscosity to trigger the autotreatment cascade.

We also plan to follow a similar approach for monitoring released pharmacological reagents. Monitoring each ligand will entail engineering different hybrid receptor-switch-galactosidase proteins as front-end sensors for these MEMS glucose-based viscometers.

We face additional technical hurdles. For instance, where should the biosensors, treatment reservoirs, and telemetry units be placed? Moreover, how will the different types of biosensors communicate, and what kind of decision-making algorithm will determine if a signal is a false alarm or true? When dealing with large implants, it may be necessary to install multiple biosensors, but where should they be placed? One model has part of a prosthesis hollowed out to contain reservoirs, but mechanical engineering constraints may mandate the use of auxiliary reservoirs placed nearby.

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