Cooper, Susan

From: Bill Costerton [costerto@usc.edu]
Sent: Friday, January 21, 2005 9:25 AM
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Subject: FW: From Paul Stoodley - Manuscript submitted to CORR


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2-14-05 not published yet

05-030

Dear Susan,

Please add this new paper to my ref list ::: Thanks

Bill

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-----Original Message-----
From: Mary OToole [mailto:MOTOOLE@wpahs.org]
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Subject: From Paul Stoodley - Manuscript submitted to CORR

Attached is the ms. "Engineering approaches for the detection and control of orthopedic biofilm infections" submitted to Clinical Orthopaedics and Related Research in Word and PDF formats. Thanks. Mary O'Toole for Paul Stoodley Center for Genomic Sciences 412-359-4707

Cover Letter

January 20, 2005

Dr. Arlen D. Hanssen
Guest Editor
Clinical Orthopaedics and Related Research
Department of Orthopedics
Mayo Clinic
Rochester, MN 55905

Dear Dr. Hanssen:

We are submitting with this letter a manuscript entitled **"Engineering approaches for the detection and control of orthopedic biofilm infectioins"** for publication in the supplementary issue of *Clinical Orthopaedics and Related Research* associated with the November Workshop on Musculoskeletal Infection sponsored by *The Association of Bone and Joint Surgeons.*

All co-authors have seen and agree with the contents of this manuscript, and the work has not been submitted or published elsewhere.

I thank you for your consideration of our request and the manuscript.

Sincerely,

Garth D. Ehrlich, Ph.D.
Executive Director,
Center for Genomic Science
Professor of Microbiology and Immunology
Professor of Otolaryngology
Drexel University College of Medicine

Editorial Manager(tm) for Clinical Orthopaedics and Related Research
Manuscript Draft

Manuscript Number:

Title: Engineering approaches for the detection and control of orthopedic biofilm infections

Article Type: Symposium/Proceedings/Workshop

Section/Category: 2004 CT Brighton/ABJS Workshop on Musculoskeletal Infection (Dr. Arlen Hanssen, Guest Editor)

Keywords: Bioelectric; biofilm; MEMs; orthopaedic implants; staphylococci; viscometer

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Manuscript Region of Origin:

Abstract:

Engineering approaches for the detection and control of orthopedic biofilm infections

Running Title: **Detection and control of biofilms**

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ABSTRACT

Artificial joints are subject to chronic infections associated with bacterial biofilms which can only be eradicated by the traumatic removal of the implant followed by sustained intravenous antibiotic therapy. We have adopted an engineering approach to develop both electrical current–based approaches to bacterial eradication, and microelectromechanical systems (MEMS) that could be embedded within the implanted joint to detect the presence of bacteria and to provide *in situ* treatment of the infection before a biofilm can form. In the former case we will examine the combined bacteriocidal effects of direct and indirect electrical fields in combination with antibiotic therapy. In the latter case bacterial detection will occur by developing a MEMS-based biosensor that can "eavesdrop" on bacterial quorum sensing-based communication systems. Treatment will be effected by the release of a cocktail of pharmaceutical reagents contained within integral reservoirs associated with the implant, including a molecular jamming signal that competitively binds to the bacteria's quorum sensing receptors (which will "blind" the bacteria preventing the production of toxins) and multiple high dose antibiotics to eradicate the planktonic bacteria. This approach is designed to take advantage of the relatively high susceptibility to antibiotics that planktonic bacteria display compared with biofilm envirovars. Here we report the development of a generic MEMS biosensor that measures changes in internal viscosity in a base fluid triggered by a change in the external environment.

INTRODUCTION

Orthopedic implant infection is a devastating disease because of the physical and emotional trauma to the patient associated with revisional surgery, compounded by the long term post-operative treatment (9, 27). Infected arthroplasties are not a rare or orphan disease state. Current rates of infection for artificial joints (over the lifetime of an implant) vary by hospital, surgeon, and study, but the best estimates suggest an infection rate of 1-2%, which produces a figure of 4-8000 infected arthroplasties requiring surgical revision annually. Implant infection is not limited to orthopedic implants; it is conservatively estimated that there are 1.32 million prosthetic devices that become infected each year in the United States alone (Table 1). The cost in dollars is enormous, as is the morbidity and patient suffering caused by these persistent infections.

Bacterial Biofilms

Bacterial biofilms can form on any artificial surface that has been introduced into the human body, as well as on tissues adjacent to the implanted surface. It is important to emphasize that artificial joints of any type (hips, knees, elbows, etc); orthopedic screws, bolts and rods are all vulnerable to hosting a biofilm (27). Implant infections can result acutely when infectious bacteria enter the implant site during surgery or recovery. However, the majority of implant infections are subacute or chronic and result from systemic seeding of the implant following a septic event. Once a biofilm is established within the body, it is nearly impossible to eradicate it - even with high doses of antibiotics (7, 10). The biofilm can also produce periodic planktonic "showers" of bacteria (i.e. bacteria shedding from the biofilm) into the blood stream which can result in episodic acute systemic infection on top of the chronic infectious nidus for the patient (10). The biofilm model introduces a novel paradigm into microbiology and infectious disease, i.e. the concept that bacteria have a life cycle just as many eukaryotes do (11). This knowledge provides us with

a framework for understanding persistence and chronicity in bacterial infections, but more importantly it provides us with a starting place for the development of new approaches to the treatment of what have heretofore been intractable infections. Like all abiotic systems introduced into the human body, arthroplasties are prone to bacterial biofilm infections and even mixed kingdom biofilm infections composed of bacterial and mycal species (Fig. 1).

Biofilm bacteria, once firmly established on a non-living surface within a host, essentially become a permanent feature of that surface. There is no means, short of removing the infected device or killing the host, to eradicate the biofilm. Biofilms are not simply collections of individual bacteria, but rather are complex co-operative communities composed of one or more species of bacteria (and/or fungi) embedded within an extracellular matrix, displaying discrete temporal and spatial organizational properties, and possessing a wide range of environmental sensing mechanisms linked to adaptive responses that operate at the population level rather than at the individual cell level. This introduces an important concept with respect to biofilm pathogenesis in that the biofilm as a whole is acting as the organism instead of the individual bacterial cell. This realization provides for a fundamental change in our consideration of the evolutionary pressures that are operative on the bacteria. This duality of existence provides for an unprecedented level of adaptability and fitness for bacterial species that can transition between planktonic and biofilm environmental variants ("envirovars").

From a medical perspective the most important aspect of biofilm bacteria is their near imperviousness to elimination by either host defense mechanisms, or even intensive long-term antimicrobial therapy. We have demonstrated that these resistances to host and pharmacological attack result in part from the fact that bacterial biofilms contain many different environmental niches with respect to nutrient availability, and pH and O2 tension. The bacteria composing the biofilm express multiple phenotypes in response to these substrate gradients (4, 23). Thus, the bacteria possess a

phenotypic plurality which ensures that some subpopulation of them will survive any type of antibiotic treatment based on metabolic considerations (7, 9, 10, 25). Moreover, the bacteria within a biofilm function cooperatively, analogous to a simple metazoan. At this stage of their life cycle, natural selection pressures will occur at the population level, as opposed to the individual cell during planktonic growth. We posit that it is this duality of existence modes which provides bacteria with their extraordinary fitness (11). In the presence of abundant nutrient sources and the absence of severe environmental threats bacteria will grow as rapid planktonic blooms, but in the face of adversity they will adapt and persist as biofilms.

In this paper we describe two engineering-based approaches for the detection and control of biofilm infections associated with orthopedic implants which can be deployed *in situ*. The economics of the treatment of individual infected arthroplasties multiplied by the large numbers of people affected by implant infections justify the increased costs associated with the development and manufacture of such "intelligent implants". Such devices would be engineered to have multi-functional capabilities including bacterial diagnostics, treatment regimens with automonitoring of dispensed pharmaceuticals, and telemetry to provide feedback regarding the microbiological and pharmacological state of the joint.

An engineering approach to prevent infected arthroplasties

We have organized a multidisciplinary biomedical-engineering approach to develop a self-diagnosing, self-treating, self-monitoring artificial joint (an "Intelligent Implant") (Fig. 2) to combat the devastating problem of post-implant bacterial biofilm infections that form on artificial joint prostheses. Towards this end a meeting (Designing Intelligent Orthopedic Implants for Biofilm Control) was held on April 10-12, 2003 at Big Sky, Montana which was attended by surgeons, microbiologists, biochemists, mechanical engineers, electrical engineers, and MEMS (microelectromechanical systems) engineers.

The most salient point made by the surgeons (with regard to implant design) was that a significant majority (> 60%) of arthroplastic infections results from two related staphylococcal species, *Staphylococcus aureus* and *S. epidermidis*. The microbiologists were united in their view that the infection should ideally be controlled prior to the formation of a biofilm as treatment regimens do not currently exist that can eradicate a biofilm on an abiotic surface implanted into human tissue. From the biosensor/MEMS engineers it was learned that the most difficult technical challenge is to overcome biofouling of an implanted device which would contain a membrane through which ligands would pass to induce a signal cascade. The electrical engineers indicated that the most problematic area for them was the need to provide power to the device for years without external leads which could become a source of infection. These consensus viewpoints, together with a preferred embodiment of what would be required in the design of an intelligent implant, were presented at the ASM Conference on Bio-, Micro-, and Nanosystems held in New York City July 7-10, 2003 (8).

The overall concept in the design of an Intelligent Implant is to produce an arthroplasty that contains a MEMS-type biosensing device that can "eavesdrop" on bacterial communication systems associated with auto-induction, quorum sensing, and biofilm formation. Most pathogenic bacterial species (as well as most nonpathogenic species) produce and respond to intercellular signaling molecules which are designed to detect either the concentration of bacteria in a given locale (quorum sensing) (20) or to determine the rate of diffusion within the ecosystem in which the bacteria find themselves (22). Depending on the bacterial species and the environment, quorum sensing serves to provide coordination of metabolic switching among a population of like bacteria so that they act in concert for the benefit of the population instead of as individual organisms. In the case of many pathogens the detection of a quorum of bacteria induces the production of virulence factors and toxins. This co-ordinate inducible phenomenon

occurring on a population level has been interpreted as a survival strategy for pathogens wherein they try to remain "below the radar" of the host's pathogen detection systems until such time as their numbers are great enough to overwhelm the host's initial response. A classic example is *S. aureus*-induced toxic shock syndrome (17).

Upon intercepting the bacterial signals, the MEMS biosensor will send a signal to a pair of integral gated reservoirs which will: 1) release inhibitory compounds that will prevent biofilm formation; and 2) release antibiotics at very high concentrations locally that will eradicate all planktonic bacteria that are in proximity to the joint prior to their establishing a biofilm. The ability to provide site-specific dosing with antibiotics would have the benefit of being able to deliver much higher concentrations of antibiotics at the locus of infection than could be tolerated by the host through systemic treatment regimens. Moreover, local dosing would potentially permit the use of highly-efficacious antimicrobials that have specific organ system toxicity profiles that makes them totally unavailable using any of our current systemic dosing regimens. Thus, not only could this strategy provide for higher dosing levels of systemically tolerated antibiotics, but it could also increase the size of the available pharmacopoeia – a significant advantage considering the high percentage of pathogenic strains that have acquired one or more planktonically-active antibiotic resistance genes.

The MEMS biosensors and the drug reservoirs would be connected to a telemetry system embedded within the prostheses which would be accessible to the patient and physician using a handheld Bluetooth monitoring device which in turn would be able to communicate with the wireless web. Thus, patients would be able to take a reading anywhere in the world and upload the data to the internet from which their physician could monitor the condition of the joint, regardless of location.

Steps towards the development of MEMS biosensor

The critical development components upon which the fate of the Intelligent Implant system hangs are the MEMS-based biosensors. This and the integration of the various modules are expected to consume the great bulk of the developmental process. As noted above, the predominant bacterial species associated with infections in artificial joints are *S. aureus* and *S. epidermidis*, which collectively account for a significant majority of all implant infections. Fortunately as these staphylococci both utilize a well-characterized peptide-based quorum sensing system which is amenable to manipulation (2). Thus, a decision was made to focus exclusively on these organisms for the development of a first generation MEMS biosensor. Both of these species produce a quorum sensing peptidyl autoinducer (ligand) termed RAP (RNA III activating protein) and a cognate cell-surface based receptor termed TRAP (target of RNA III-activating protein) which becomes activated through phosphorylation upon binding RAP. TRAP activation triggers upregulation of a secondary two-component cell-signalling system encoded by the *agr* locus. Activation of *agr* results in production of AIP (autoinducing peptide) which is produced by cleavage of the prepeptide AgrD by the AgrB protein and AgrC, its cognate receptor encoded within the *agr* locus. Binding of AIP to AgrC initiates a phosphorylation cascade which results in up-regulation of RNA III synthesis from the *agr* locus. RNA III is a central pleiotropic regulator that controls the expression of numerous virulence factors. The *agr* locus contains divergent transcriptional systems controlled by the promoters P2 and P3 which encode RNA II and RNA III, respectively. P2 is activated by the RAP-TRAP system and P3 by the AIP-AgrC system. The first MEMS biosensor is being developed to detect the earliest stage of Staphylococcal intercellular communication (RAP-TRAP) to provide the greatest lead time prior to biofilm formation for treatment.

Interference with the RAP-TRAP signaling system by the peptide RIP (RNA III inhibiting peptide) has already been demonstrated to produce a beneficial effect in terms of reducing staphylococcal-based

pathogenesis (3). RIP is an octapeptide that is synthesized by *S. warnerii* and *S. xylosus,* both coagulase-negative staphylococci. Both native and synthetic forms of RIP have been demonstrated to competitively inhibit RAP's binding to TRAP, making RIP highly effective in inhibiting RNA III synthesis *in vitro* and suppressing pathogenesis *in vivo* (2, 15). Moreover, mice vaccinated with RAP show protection from challenge with *S. aureus* in direct correlation with their titers of anti-RAP antibodies (14). Thus, we have chosen the RAP-TRAP system as a validated target for the initial focus of our MEMS-based biosensor development, with the objective of creating a biosensor based on TRAP which would detect bacterially-produced RAP (Fig. 2).

RAP's binding to the biosensor, just as in the bacteria, would result in a confirmational switch in a chimeric TRAP molecule that would activate an enzymatic moiety triggering a signal transduction cascade within the MEMS device. This cascade would result in the release of RIP and anti-RAP antibodies from the implant's reservoirs. The three-dimensional space proximal to the prosthesis would then be flooded with a bivalent bolus of bacterial blinding agents that would prevent toxin production, biofilm development and quorum sensing by the planktonic staphylococci present in the area. Simultaneously, a second set of reservoirs would release a cocktail of potent antibiotics including, for example, nafcillin and perhaps vancomycin to kill the planktonic staphylococci. The release of the various specific signaling inhibitors and antimicrobials would, in turn, be monitored by a second set of MEMS-based sensors to ensure that an adequate release had occurred *in situ*. Finally, all the activity of the various biosensors and reservoirs would be stored within a memory module embedded in the implant that would be available for uploading upon signaling from a remote hand-held unit that would be provided to the patient.

Development of a Generic Biosensing Core

For design simplicity and cost control it would be advantageous to have a single generic MEMS-based biosensing core which could be 'easily' modified to produce a family of biosensors each with a unique specificity. Toward this end we have chosen to work on the development of a generic MEMS device that will detect an internal change in viscosity. In this model system the sensor is actually a cantilever-based viscometer. We have successfully constructed a microviscometer sensing unit (Fig. 2) by adapting a system developed by Jeckelmann and Seibold (www.disetronic.com/download/02_Seib.pdf) for monitoring the blood glucose levels of diabetics. In their system (non-MEMS) changes in viscosity are measured based upon competition between glucose and dextran (a glucose polymer) for binding with the quadravalent Concanavalin A (ConA) molecule. In the absence of free glucose, four large dextran molecules will bind to each molecule of ConA producing a high viscosity gel-like matrix. If glucose enters the system then it will compete for the ConA binding sites resulting in a drop in viscosity. The MEMS-type viscosity biosensor which we developed works by measuring the deflection of a cantilever in inverse proportion to the viscosity (Fig. 3). Thus, the greater the deflection of the cantilever the lower the viscosity and the greater the signal recorded by the system. For the device we are designing enzymatic glucose production (generated from a polysaccharide) will be the signal that the TRAP-based biosensor has been triggered by RAP binding.

As mentioned above, in an effort to circumvent the universal problem of biofouling that befalls all implantable biosensors we have developed a strategy in which we will not attempt to pass the ligand to be detected (RAP) into the biosensor. Instead we will attempt to transduce a signal into the biosensor using a transmembrane-based conformational protein switch, in essence mimicking the natural process by which most cells receive information about their environment. To accomplish this we are taking a protein engineering approach to the problem in which the extracellular domain of TRAP will be fused to a

transmembrane conformational switching domain that in turn is fused to a glucosidase. When the hybrid TRAP molecule is unbound the glucosidase on the interior of the biosensor will be in an inactive conformation and the biosensor will remain in high viscosity status, indicating that there are no bacteria nearby. However, if staphylococcally produced RAP is present in the vicinity of the joint, it will bind to the engineered TRAP proteins on the surface of the biosensor and the molecular switch will trigger activation of the glucosidase moiety. The activated glucosidase will release free glucose from a substrate glucose polymer which will, in turn, competitively bind to the concanavalin A, displacing the dextran and resulting in a decrease in viscosity which will initiate release of the multi-component treatment regimen described above.

A similar protein engineering approach will be used to monitor the concentration of the released pharmacological reagents. For each ligand to be monitored a hybrid receptor-switch-glucosidase protein will be engineered to function as a "front end" for the MEMS glucose-based viscometer. Thus the most difficult developmental process associated with this project will serve multiple masters.

Technical challenges to implementing a MEMS-based biosensor

The number and placement of the biosensors, treatment reservoirs and telemetry unit(s) is far from established. Additionally, the communication between the multiple biosensors and among the several types of units must be developed and fitted with an intelligent decision making algorithm that can determine if a signal is likely a false alarm or a true "call to arms". Moreover, the operating system must be capable of deciding between a local release from a single reservoir or a global release from all reservoirs. Due to the size of the implants, it is felt that multiple biosensors should be employed providing as broad an area of coverage for the prosthesis as possible. It may not be possible, however, to place the sensors on the articulating surfaces and we don't know if it will be efficacious or even possible

to position biosensors on the parts of the implants which are seated directly in existing bone. One model has part of the prosthesis hollowed out for reservoirs, but mechanical engineering constraints may mandate the use of auxiliary reservoirs which would have to be placed with the implant. Two other significant challenges which must be overcome are: 1) designing a system that will be stable and functional for an extended period of time *in situ* at 37 °C; and 2) resetting the biosensor to baseline after exogenous signal is no longer present. It is understood that these and other major developmental hurdles will be have to be overcome at all levels and stages of this project.

The Bioelectric Effect

In addition to delivery of antibiotics to the site of infection we are also designing ways of increasing antibiotic efficacy against biofilms using the "bioelectric effect". The bioelectric effect is the synergistic killing effect observed on biofilm cells that are exposed to antibiotics in the presence of an electric direct current (DC) or alternating current (AC) field. The use of electric currents and electromagnetic fields to modulate biological processes has become an increasingly popular subject of scientific enquiry in recent years. Although many such studies are still in their infancy, in orthopedics at least one avenue of investigation has shown substantial progress: the use of electromagnetic stimulation to promote healing of problematic bony fractures. Aaron et al. (1) discuss the results from several trials that indicate electromagnetic fields can be used to accelerate bone formation and healing. Nelson, et. al. (18) discuss pulsed electromagnetic fields, capacitive coupled fields (electric fields rather than magnetic fields are used to induce currents), and low-intensity ultrasound as methods that are used to stimulate bone healing and bone formation. Cell studies are reported by Guerkov, et. al. (12). Their results suggest a cascade of regulatory events is stimulated by the pulsed electromagnetic fields in the human hypertrophic and atrophic nonunion tissues. Ryaby (24) presents a review article on the clinical use of electric and

electromagnetic fields that are being used in the clinic to assist fracture healing, and a review paper by Otter et. al. (19) covers a number of the applications of electromagnetic fields to the field of bone healing.

With this background of practical success, efforts are now also underway to determine whether deployment of electric currents and fields against biofilms can become an effective therapy against infection. Early reports in *in vitro* systems have been encouraging, most particularly in observing the success of the bioelectric effect in potentiating the efficacy of concomitant antibiotic action against biofilm bacteria (Table 2). Costerton et al. (6) reported that application of a low-intensity direct current (max. 2.1 mA/cm^2) to a flow cell in which *Pseudomonas aeruginosa* biofilm had been established was relatively ineffective in reducing bacterial numbers alone, but increased the killing efficacy of tobramycin by over 4 orders of magnitude when the two modalities were applied concurrently. The concentration of antibiotic necessary to treat biofilm bacteria was thus effectively reduced to within the same order of magnitude as that required to achieve MBC values in planktonic bacteria, rather than the thousand-fold or more increased concentrations typically required to kill biofilms. Importantly, this increased efficacy brings antibiotic doses down to physiologically and clinically acceptable levels.

These results were confirmed by McLeod et al. (17), who again found that direct electric current potentiated the action of tobramycin against Pseudomonal biofilms by an almost thousand-fold reduction in bacterial viability in an exposure chamber system (Table 2, Fig. 4). McLeod and co-workers, however, kept current constant for the full duration of treatment and were thus able to compile a dose-response curve for current efficacy, with the lowest optimal current value appearing at 1 mA. Similar encouraging results have been noted for the bioelectric effect against *Klebsiella pneumoniae* treated with tobramycin, *Staphylococcus epidermidis* treated with tobramycin, *Streptococcus gordonii* treated with gentamicin, and *Candida albicans* treated with cycloheximide (13, 28).

Caubet et al. (5) have evaluated the use of both DC and a ten megahertz (10 X 10^6 hertz) time-varying AC in an exposure chamber similar to that of McLeod, et. al. (16). These authors used *Escherichia coli* biofilms treated with either gentamicin or with oxytetracycline and studied the effect of adding either a DC or the ten megahertz AC to the antibiotic in the support medium. The current for both the DC and AC work was delivered through the support medium flowing in the exposure chamber via electrodes extending into the medium at either end of the exposure chamber (i.e. the AC was not induced in the chamber). The DC plus antibiotic treatment produced a four to five $\log_{10}$ reduction in colony forming units (CFU), achieving very similar results to those of McLeod et al, whereas a slightly more modest reduction in CFU of 2.5 to 3.5 logs was achieved with the AC plus the antibiotic. This study supports the hypothesis that enhanced efficacy can be achieved for an antibiotic with the addition of an AC, though the magnitude of the improvement may be less than that obtained with direct current.

A recent paper by Pickering et al. (21) expands the field even further by examining the effect of an induced current on antibiotic efficacy against biofilm bacteria. In this work a pulsed electromagnetic field (PEMF) was applied to *S. epidermidis* biofilm bacteria which induced a current within the biofilm. This induced current reduced the minimum inhibitory concentration of gentamicin by at least 50%, but did not show any significant effect with vancomycin.

Although there is an increasing body of evidence in support of a bioelectric effect against biofilms, the mechanism by which these electrical phenomena exert their actions is unknown, and indeed may well vary with the type of electrical current applied. In the 10 MHz AC system of Caubet et al.(5), for example, the authors point out that "there is no transport of ions between the electrodes, no creation of new ions, and no electrolysis" as would occur in a DC system, yet a bioelectric effect still appears. Stewart et al. (26) investigated the mechanisms by which a DC bioelectric effect may operate. Their work discounted the suggestions that reduced pH, increased temperature, or generation of inhibitory ions or

reactive oxygen intermediates were the relevant means by which a bioelectric effect manifests. They did find that electrolytic generation of oxygen (thus potentially increasing the local oxygen concentration in the biofilm microenvironment) appeared to partially explain the augmentation of antibiotic efficiency. Based on this study we hypothesize that the bioelectric effect results from increased metabolic and replicative activity associated with increased O_2 tensions within the biofilm bacteria which make them more susceptible to antibiotic-induced killing. It is well established that antibiotics are much more effective against rapidly metabolizing and dividing bacteria than they are to metabolically quiescent bacteria, and one of the limiting nutrients within the core of the biofilm is O_2. We have recently demonstrated that the provision of O_2 deep within the biofilm results in greatly increased metabolism (10)

The majority of work evaluating the bioelectric effect has to date been carried out in *in vitro* systems and has focussed mainly on aminoglycoside antibiotics. A single report noted above, Pickering et al. (21) failed to find any significant bioelectric effect with vancomycin but did not evaluate it in a directly applied DC system. In clinical terms for orthopedics, the bioelectric effect could become important if it can be demonstrated in *in vivo* conditions and with antibiotics routinely used against the Staphyloccoci, especially vancomycin. We are therefore establishing protocols in which direct or induced currents can be delivered within the joint capsule of an infected knee prosthesis in both small animal and large animal models. If the bioelectric effect can be achieved *in vivo*, the likelihood of developing a human-use device to supply adjuvant electrical therapy to patients with infected implants will be substantially increased.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Allegheny Orthopedic Research Foundation; funding from Allegheny Singer Research Institute; and by grant #s 3R01 DC02418 (GDE), 2R01 DC04173 (GDE) and R01 DC05659 (JCP).

LIST OF TABLES

Table 1. A compilation of prosthetic devices and the risk of infection associated with each. Data in the table were prepared from a variety of sources by the Montana State University Center for Biofilm Engineering.

Table 2. Compilation of data demonstrating the use of the bioelectric effect to increase antibiotic efficacy against various biofilms. Abx: antibiotic; DC: direct current; AC: alternating current.

LIST OF FIGURES

Figure 1. Intraoperative photograph taken during excisonal surgery to remove an infected prosthetic joint demonstrating a bacterial biofilm on an infected arthroplasty. The white material is pus that contains huge numbers of microorganisms and host-derived leukocytes.

Figure 2. Basic concept of sensing mechanism to identify the attachment of staphylococci to orthopedic implants. The unit will be flush mounted into the implant. A) Cantilever viscometer positioned within a micro chamber I filled with a high viscosity glucose polysaccharide and a dextran gel cross-linked with Con-A. B) The bacterial signature molecule (i.e. in this case RAP, blue ovals) binds to an engineered membrane containing specific receptors (i.e. TRAP, green "U"). The configurational change cause an

enzyme such as galactosidase to become activated and the glucose polysaccharide is degraded into monomers. The glucose displaces Con-A resulting in uncross-linking of dextran. The sensor detects the corresponding reduction in viscosity. D) Actual viscometer and scale.

Figure 3. The viscosity (measured as amplitude) of the dextran-Con-A hydrogel as a function of glucose concentration measured by the microviscometer.

Figure 4. Schematic showing main components for *in vitro* testing of the bioelectric effect. The biofilm is grown or positioned in the exposure chamber. Antibiotics can be pumped into the chamber with nutrients and a DC current can be applied through an anode and cathode. Adapted after McLeod et al. 1990.

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Table 1. Device-related infections and infection risk of various prosthetic devices in the United States. A compilation of and the risk of infection associated with each. Data in the table were prepared from a variety of sources by the Montana State University Center for Biofilm Engineering.

Device	Usage/Yr	Infection Risk (%)
Central venous catheters	5 million	3-8
Bladder catheters	Tens of millions	10-30
Prosthetic heart valves	85,000	1-3
Vascular grafts	450,000	2-10
Cardiac pacemakers	400,000	1-5
Cardiac assist devices	700	50-100
Penile implants	15,000	2-10
Joint prostheses	600,000	1-3
Fracture fixators	2 million	5-10
Dental Implants	2 million	5-10

Table 2. Compilation of data demonstrating the use of the bioelectric effect to increase antibiotic efficacy against various biofilms. Abx: antibiotic; DC: direct current; AC: alternating current.

Strain	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*K. pneumoniae*	*E. coli*	*S. gordonii*
Antibiotic	**Tobramycin**	**Tobramycin**	**Tobramycin**	**Tobramycin**	**Gent / Oxytet**	**Gentamicin**
Reference	**Costerton et al.**	**McLeod et al.**	**Wellman et al.**	**Wellman et al.**	**Caubet et al.**	**Wattanakaroon et al.**
Abx alone	1.53	2.88	0	0	2.11 / 1.9	0.84
DC alone	0.81	0.65	ND	2.45	0.91	1.9
AC alone	ND	ND	ND	ND	0.5	ND
DC + Abx	6.02	5.77	6.7	3.9	4.27 / >5.15	4.3
AC + Abx	ND	ND	ND	ND	3.43 / 2.80	ND

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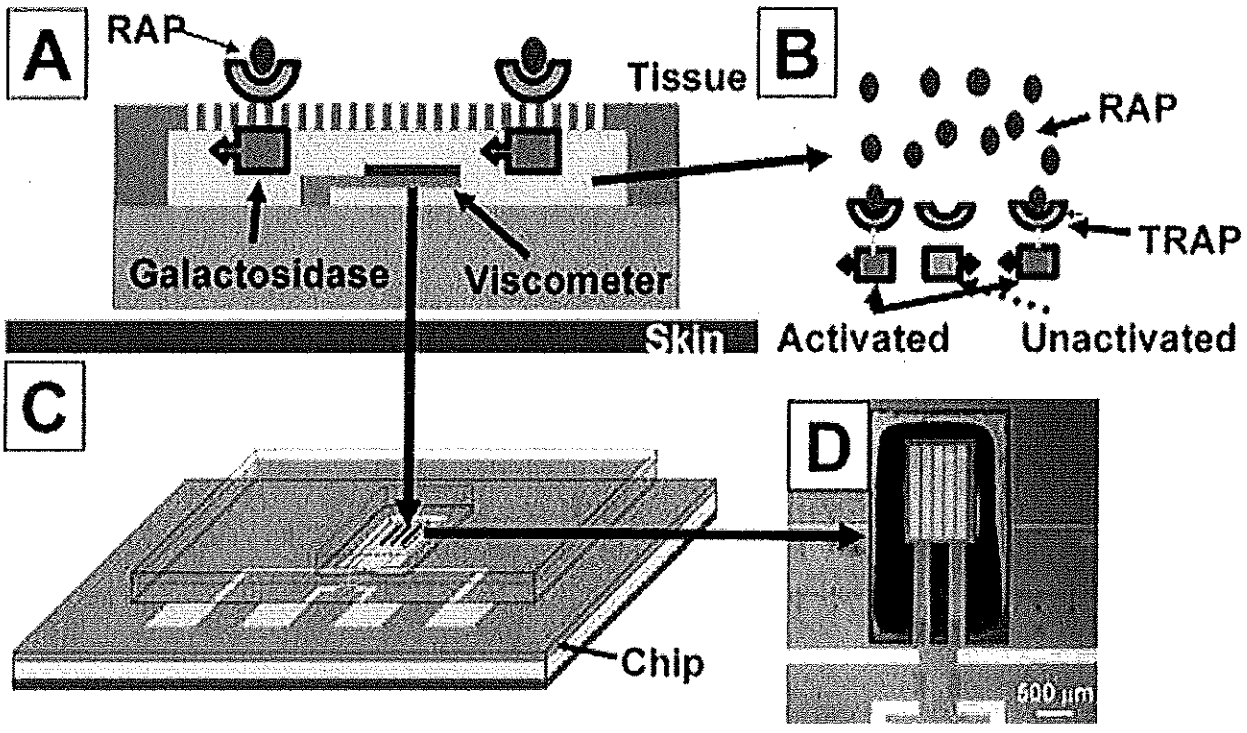

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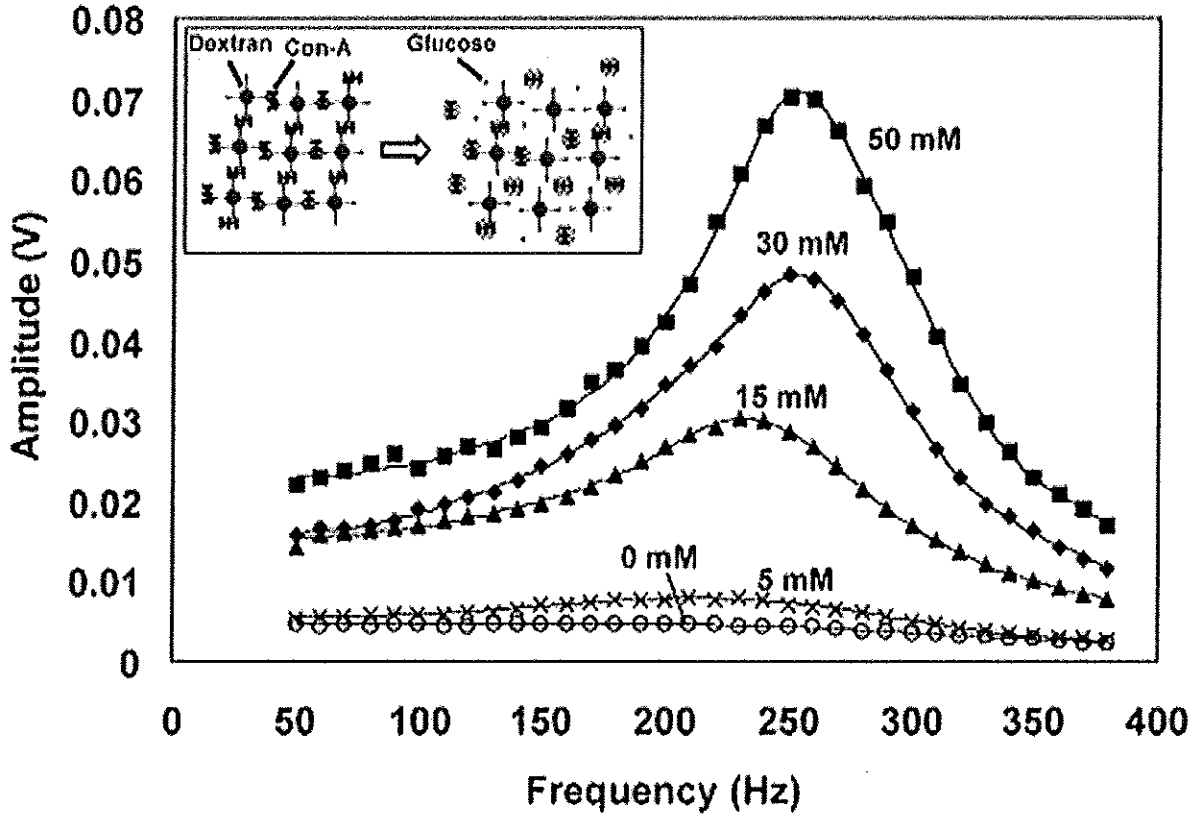

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