DESIGN AND OPTIMIZATION OF PARAMETERS AFFECTING THE PERFORMANCE OF AN ANTIBACTERIAL SYSTEM USING ELECTRICALLY ACTIVATED METALS

A Thesis in
Industrial Engineering
by
Charumani Charumani

© 2008 Charumani Charumani

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

December 2008
The thesis of Charumani Charumani was reviewed and approved* by the following:

Richard A. Wysk  
Professor and Chair of Industrial Engineering  
Thesis Adviser

Robert C. Voigt  
Professor of Industrial Engineering

Richard J. Koubek  
Peter & Angela Dal Pezzo Department Head Chair  
Department Head of Industrial Engineering

*Signatures are on file in the Graduate School
ABSTRACT

Infections associated with residual hardware devices (RHD) are becoming a challenging problem for the medical industry. Treatment of the residual hardware device infections usually involves lengthy and painful procedures. Antibiotic resistance developed by the pathogens over time is another concern. It has become essential to search for alternative antibacterial treatments to mitigate the effect of these infections. Some of the transition metals of group 11 to 14 of the Periodic Table have been investigated for their antibacterial efficacy and the bacterial resistance, and in particular silver ions have been identified as the most effective antibacterial agent. However there is no established delivery system reported which can ensure the delivery of silver ions to the site of infections associated with implanted devices.

This study examines an engineered system using silver ions to create an antibiotic environment that can significantly reduce RHD associated infections. The key is to continually generate silver ions in local concentrations allowed inside the human body so that long-term microbial control can be achieved. A brief review of residual hardware device infections, the use of antibiotic silver ions, and the concept behind such a system is followed by a summary of the \textit{in-vitro} tests, exploring the design constraints and working of the proposed system. The performance metric of the system and the variables affecting it have been identified for the prophylactic action of silver ions system. An experimental design is also presented to evaluate the parameter space of the variables affecting the performance of the system.

The Kirby-Bauer agar gel diffusion techniques were used to evaluate the bactericidal efficacy of the silver ions system against \textit{S. aureus} bacteria. In addition, the issues of current and ionic concentrations were studied including device amperage, surface area of cathode and anode, as well as the separation distance between anode and cathode. Anodic devices performed better and the current in the system and the surface area of the anode were identified as most important variables affecting the device performance. This new system allowed ionized silver to travel through media containing microbes, thus attacking the bacteria directly. The system tested demonstrated an unparalleled inhibition of the growth of microbes.
# TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................ vi

LIST OF TABLES .......................................................................................................... vii

ACKNOWLEDGEMENTS .............................................................................................. viii

Chapter 1: INTRODUCTION
  Introduction .................................................................................................................. 1
  Background .................................................................................................................. 3
  Summary ...................................................................................................................... 10

Chapter 2: LITERATURE REVIEW
  Osteomyelitis ............................................................................................................ 11
  Osteomyelitis and Prosthetic Devices ...................................................................... 12
  Treatment of Osteomyelitis ...................................................................................... 13
  Antibiotic Resistance .............................................................................................. 14
  Bactericidal Silver .................................................................................................... 15
  Pharmacodynamics of Silver and Health Effects .................................................... 15
  Silver Ions and Uses ............................................................................................... 17
  Silver Resistance ..................................................................................................... 21
  Summary ................................................................................................................... 23

Chapter 3: WORKING PRINCIPLE AND LIMITING CONSTRAINTS
  Working of the System ............................................................................................. 24
  Design Constraints ................................................................................................... 25
  Performance Measure of the System ..................................................................... 27
  Parameters Affecting Performance ........................................................................ 28
  Mathematical Model for System Performance ...................................................... 29
  Summary ................................................................................................................... 30

Chapter 4: MATERIALS AND METHODOLOGY
  System Design .......................................................................................................... 31
  Experimental Design ............................................................................................... 31
  Materials and Methodology ................................................................................... 33
  Summary ................................................................................................................... 36

Chapter 5: RESULTS AND STATISTICAL ANALYSIS
  Effect of Circuit Polarity .......................................................................................... 37
  Effect of Amperage .................................................................................................. 38
  Anode-Cathode Separation .................................................................................... 41
  Surface Area of the Anode ..................................................................................... 44
  Statistical Testing .................................................................................................... 46
  Summary ................................................................................................................... 49
Chapter 6: CONCLUSIONS AND RECOMMENDATIONS
Conclusions.................................................................50
Recommendations for Future Work..................................................51

References..........................................................................................53

Appendix A
Table A.1 Effect of varying device current on inhibition zone area..............61
Table A.2 Effect of varying separation on inhibition zone area......................61
Table A.3 Effect of varying anode area on inhibition zone area...................61

Appendix B
Table B.1 Transformed data to perform linear regression .........................62
Table B.2 Residuals and Fits values for the regression analysis....................62
Figure B.1 Residual plots of zone area obtained from the regression analysis....63
Table B.3 Residuals and Fits values of regression using interaction terms........63
LIST OF FIGURES

Figure 1: Adherence of S. aureus to bone screw...........................................13

Figure 2: Interactions of silver with micro-organisms.....................................20

Figure 3: Electrically stimulated silver releases ions carried in a bacteria-rich environment to complete a circuit.................................................................25

Figure 4: Schematic describing the geometric parameters of the device..............29

Figure 5: Schematic of the design................................................................31

Figure 6: Anodic device design as used for the testing.......................................33

Figure 7: Schematic showing proper placement of small holes in the Petri dishes......34

Figure 8: Schematic of a set up incorporating battery and resistor with metal wires.....36

Figure 9: Zone clearing for anodic and cathodic devices of 1.5A.........................38

Figure 10: Effect of current on microbial inhibition zone area............................39

Figure 11: Relationship between the inhibition zone area and log(current)..........40

Figure 12: Change in resistance with electrode separation.................................42

Figure 13: Inhibition zones produced by using different separations..................43

Figure 14: Effect of surface area on one of inhibition.....................................45

Figure 15: Inhibition Zone with anodic device..............................................48
LIST OF TABLES

Table 1: Range of values of separation, current and anode length........................32
Table 2: Table for effect of current........................................................................39
Table 3: Total resistance in the circuit and its effect on the zone of inhibition.........42
Table 4: Table for Effect of Surface Area...............................................................44
Appendix A........................................................................................................61
Appendix B........................................................................................................62
ACKNOWLEDGEMENTS

Many people have helped me in my Masters over the course of the last two years. I would like to express my sincere gratitude to my adviser and professor, Dr. Richard A. Wysk, who introduced me to this area of research. Throughout my Masters, his encouragement and constant support were indispensible.

I am grateful to Dr. Robert C. Voigt, for his help and support for my research as well as my professional development. I greatly appreciate his taking the time to critically review this Masters thesis.

I would also like to acknowledge Dr. Bhushan M. Jayarao, who permitted me to work in his lab and provided useful input. I also thank Dr. Mary J. Kennett and Thomas A. Fuller for their valuable suggestions during my research.

I am grateful to my colleagues and friends, Rachel Abrahams, Amit Arora, Gaurav Bhardwaj, Kokonad Sinha, Amit Kumar, Rituraj Nandan and Rohit Rai for their support and for making my stay at Penn State a memorable one.

Last, but not the least, I thank my family. My father Mr. Jatan Singh, my mother Mrs. Promila Rani, my aunt Miss Suman, my lovable sisters Mrs. Pragya Mansi and Miss Divya Singh, and my adorable niece Pihu. This would not have been possible without your love and support.
To my parents
Mr. Jatan Singh, Mrs. Promila Rani and Miss Suman.
Chapter 1

This chapter introduces the problems associated with the residual hardware devices, addressing their background, nature and impact. Infection rates due to residual hardware devices (RHD) and the major causes of these infections are examined along with the cost associated with these RHD infections. The relative ease with which bacterial species adhere to residual hardware as well as the relative disadvantages of the body to combat the infection associated with residual hardware are discussed. Also background information into the prevention and treatment of residual hardware devices infections is summarized. It examines the metal ions which can be used as bactericidal agents and in particular identifies the properties and scope of silver ions to prevent infections. The chapter concludes with a concept design for a system to provide controlled delivery of silver ions for residual hardware devices to the site of infection.

Introduction

Medical prosthetic devices, particularly joint replacement and fracture fixation devices are an indispensable component of modern medical treatment. According to the Canadian Institute of Health report of 2002, over the six-year period between 1994 / 1995 and 1999 / 2000, the total knee replacement surgery rate rose by 33.1%, from 50.5 to 67.2 per 100,000 people while the total hip replacement surgery rate rose by 8.5% from 55.0 to 59.7 per 100,000 people [Canadian Institute for Health Information, 2002]. In 2004, it was estimated that approximately 600,000 joint prosthesis and 2,000,000 fracture-fixation devices were inserted into patients in the United States [Cornell, 2004]. Joint replacements and fracture stabilization / fixation are becoming more and more common surgical techniques, due in part to the advancement in surgical procedures and medical devices. Patients receiving the joint replacements and fracture fixations device are living longer due to a healthier lifestyle. Advances in surgical methods have led to a lower morbidity and mortality rate associated with implant surgeries. Although the overall rate of post-operative infections has decreased the total number of RHD associated infections is increasing. Any person with a piece of residual orthopedic equipment within the body
is at a high risk of RHD related infection. This is due in part to the fact that some biomaterials, like the ones used for prosthetic joint implants or fracture fixation devices provide an excellent adhesive surface for bacteria. Many bacteria adhere to prosthetic devices exceptionally well due to specific modifications of their external structure, thus requiring a smaller than usual bacterial inoculum to produce a severe infection.

In the US, every year there are approximately 2 million cases of nosocomial infections. Half of these are associated with indwelling medical devices such as catheters, fracture fixation devices and joint arthroplasties [Darouiche 2001]. The approximate incidence of infection for approximately 2,000,000 fracture fixation implantations in 2004 was 5%. These infections have major clinical and economic consequences. Infections associated with cardiovascular implants have a higher mortality rate and infections with orthopedic devices often result in permanent disabilities and as well as have a high mortality rate. The annual cost to mitigate these infections was approximately $1.5 billion in 2004 [Darouiche, 2004; Ehrlich, 2005]. For joint prostheses alone, an approximate incidence of 1-2% infection in 600,000 arthroplasties in 2004 resulted in an estimated infection mitigation cost of $360 million. Such high costs reflect the difficult and lengthy course of treatment for RHD-associated infections.

Patients suffering from osteomyelitic RHD infections must undergo difficult and costly treatments that include extended hospitalization, local debridement of the infected area, aggressive antimicrobial therapy, device removal and often staged total joint replacement. Total elimination of the osteomyelitic infection is usually achieved only after device removal followed by an aggressive six week course of immobilization and aggressive antimicrobial therapy. The number of doctor visits for an infected implant patient is at least six times the number of visits by a non-infected implant patient. The infected implant patients are subject to three times as many operations and twice as many radiographic examinations as the non-infected patient. The treatment cost of the infection is approximately 5.23 times as much as a non-infected implant [Bengston, 1993]. Besides the complicated and expensive treatment, these infections can also hinder the normal
working of the patient. This emphasizes the need for an economical, quicker and safer means to combat residual hardware infections.

**Background:**

To understand the inherent risk associated with residual hardware devices, it is important to understand that orthopedic implant surfaces required to promote bone adhesion also provides effective surfaces for the bacteria to seed and grow. Thus a patient who has a residual hardware within the body is at a high risk of infection. RHD-associated infections result from interactions between host and microorganisms, and concomitant factors related to implant surfaces whether they are metallic, latex, silicone, or other forms [Arciola, 2005; Donlan, 2005; Schierholz, 2001]. When in contact with the metal matrix, bacteria achieve adherence by expressing “adhesives” on their surface membranes with host plasma components within the blood [Kochwa, 1977]. Adhesions are surface proteins which are embedded within the cell wall of the bacteria. In vitro studies [Vaudaux, 1989 and 1993] have shown that plasma proteins such as fibronectin, fibrinogen and vitronectin strongly promote bacterial adhesion to the polymeric and metal surfaces.

An infection of the bone is termed as osteomyelitis. Osteomyelitis is difficult to treat not only due to the nature of the bacteria, but also because of the natural reaction the body undergoes to combat the local infection and the structure of the bone. Disruption of epithelial and mucosal barriers and tissue trauma during device installation simultaneously trigger host immune responses and impair host defense mechanisms [Schierholz, 2001; Vinh, 2005]. Once installed, conditioning films composed of host cells and cell products coat implant surfaces and often facilitate microorganism adhesion and colonization [Donlan 2002]. Also the micro channels of the bone make it difficult for the antibiotics to gain access to the infection, but allow bacteria to proliferate and spread to different areas of the bone and surrounding tissue. Frequent sources of acute infection are opportunistic microorganisms present on epithelial surfaces. Surface seeding of RHDs may occur during installation, as a result of microorganisms traversing incision sites,
migration along catheter surfaces, or via systemic spread following a septic condition [Schierholz, 2001; Ehrlich, 2005]. Species frequently associated with RHD infections include: *Staphlococcus aureus*, methicillin-resistant *Staphlococcus aureus* (MRSA), *Staphlococcus epidermidis*, *Enterococcus faecalis*, *Eschericia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Although not all colonized RHDs become infected, those that do involve biofilm formation [Darouiche 2004]. Biofilms or glycocalyx layers are complex sessile microcolonies of bacteria or yeast embedded within a microbial-derived protective extracellular polymeric matrix [Ehrlich, 2005]. The glycocalyx enables certain bacteria to resist phagocytic engulfment by the white blood cells in body, in addition to aiding to adhere, colonize and resist flushing. Once a biofilm forms, it is virtually inseparable from the implant.

Though advances in medical therapy and sterilization technique combined with advanced antibiotic therapy have decreased the rate of RHD infections to 1% - 2% in 2004, treatments for chronic infections (those involving biofilms) are rigorous due to the protective nature of the implant [Darouiche, 2004]. Since the orthopedic implant impedes the native blood blow, antibiotics transported through the blood stream do not reach the infected site efficiently, resulting in a formidable antibiotic delivery barrier [Schierholz, 2001]. Moreover, the increasing resistance expressed by the bacteria renders the usage of antibiotics less effective. The increased difficulty to kill the bacteria is due to the nosocomial nature of these infections and the continual exposure to antibiotics and disinfectants used in the hospital making them extremely difficult to eliminate. Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections [Darouiche, 2004]. When a person takes an antibiotic, the drug kills the defenseless bacteria, however, if there is a bacteria which has developed resistance against the action of the drug, it is left behind. These renegade bacteria then multiply, increasing their numbers a million-fold in a day, becoming the predominant microorganism. A bacterium that expresses resistance to a given antibiotic will not be killed by the antibiotic. Thus it is foreseeable that curing these infections may become an even more serious issue in the future [Ehrlich, 2005].
Traditional antibiotics or combinations thereof have limited applicability to the resistant strains of osteomyelitic infections associated with RHDs. New anti-bacterial methods are needed to combat these infections. Moreover, a method of treatment that does not require removal of the prosthetic implant or hardware device would be extremely beneficial to both the patient and physician. There is a current need for a non-antibiotic or alternative antibiotic means of treating infections that will remain a cost effective strategy when compared to traditional approaches. Historically transition metals, particularly those in columns 11 through 14 of the Periodic Table have been used to treat microbial infections. Gold, silver and copper have been used frequently in medical use as components of wound dressings, external skin treatments, and debridement agents for hundred of years with anecdotal evidence of antimicrobial efficacy [Gager, 1935; Hill, 1939; Klasen, 2000 A and B]. Many of these elements contained in columns 11 -14 of the Periodic Table are potentially toxic to cells. However if engineered properly the controlled elution of these elements will remain below threshold toxicity values for local cells, allowing the cells to metabolically compensate for the toxic element. By properly engineering a control mechanism the desired antimicrobial effect can be achieved while remaining locally non-toxic to mammalian cells.

In 1992, Mader determined that, in most cases the antimicrobial efficacy of a metallic substance, particularly silver, is related to the ionic form of the metal. [Mader, 1992]. The surfaces of metals in air or aqueous solutions give off metal ions naturally but often at very low rates. The inherent ability of a metal to form a reactive ion is termed the anodic potential of the metal and varies for each metal. Noble metals such as gold, silver and copper behave very cathodically when in “electrical contact” with other metals as indicated by a relatively positive standard aqueous electrode potential (a measure of amount of the anodic (-) or cathodic (+) potential of a metal). A positive standard aqueous electrode potential, like that associated with the noble metals, indicates that these metals do not readily ionize unless a forcing electrical potential is applied. The number of ions generated by passing a given current across the metallic surface is described by
Faraday’s law. Faraday’s law suggests that under ideal conditions the number of ions generated per period of time is directly proportional to the amount of electrical current applied to the surface. If proper electrical stimulation is provided the evolution of ions from the surfaces of noble metals can be controlled. Without such a forcing current, noble metals such as gold, silver and copper will not generate a significant amount of metal ions and are therefore almost inert [Mietzner et al, 1997].

Mader [Mader, 1992] provided the understanding and rational behind the clinically limited use of metallic species as antimicrobial treatment. Effective treatment of microbes is only produced by properly controlling the microbial toxic form of the element, the ion. Ionization enhances the inherent antimicrobial activity of certain metal and increases their efficacy for treating infections. However many metals are toxic to the human body and the ionic form of the metals are no different. If heavy metal species are to be used to combat infection, proper control needs to be established. Metal ions need to be delivered to the site of the infection in concentrations that are locally toxic to the bacterial species but remain below total body toxicity levels. Throughout history researchers have demonstrated efficacy of heavy metals for bacterial elimination but have not been able to engineer mechanisms that allow for precise delivery and control of the potentially toxic species.

A study conducted by Fuller [Fuller 2005] identified the metals or metallic ionic forms within Periodic Table columns 11 through 14 that could potentially serve as antimicrobial agents. The study measured the zones of inhibition that resulted after a 24 hour incubation period. Using a method adopted from the Kirby Bauer agar gel diffusion technique, the bacteriocidal / bacteriostatic efficacy of eight metals (copper, titanium, gold, silver, cadmium, nickel, zinc and stainless steel AISI 316L) and their electrically generated ionic forms were tested against 5 bacterial species and one fungus commonly associated with osteomyelitis. The gram positive bacterial species included: Staphylococcus aureus, Enterococcus faecalis, and methicillin resistant Staphylococcus aureus (MRSA). The gram negative species included Escherichia coli and Pseudomonas aeruginosa. Candida Albicans was the only fungal species tested. The results of this
study indicate that if an antimicrobial effect is to be achieved from the ionized metal within those tested, the metal will be copper, silver or cadmium. However, gold, titanium, zinc, nickel, and stainless steel did not inhibit growth of any bacterial species at any current levels. Cadmium and silver produced inhibition patterns across all electrically generated current levels. This study also indicated that ionized copper inhibited only the Gram [+] bacteria. Cadmium, due to the toxicity profile associated with in vivo use of the metal may not make a suitable RHD associated metal [Agency for Toxic Substances and Disease Registry (ATSDR) 1999].

Silver ions appears to have efficacy against a broad range of bacterial species [Fuller 2005], so treating antimicrobial-resistant bacteria as well as common species with ionized silver could produce substantial benefit in terms of cost savings and patient well-being. The antimicrobial action of silver or silver compounds is proportional to the amount of bioactive silver ions (Ag\(^+\)) released and its availability to interact with bacterial or fungal cell membranes. The silver ion is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes. Bacterial (and probably fungal) sensitivity to silver is genetically determined and relates to the levels of intracellular silver uptake and its ability to interact and irreversibly denature key enzyme systems. On a cellular level, silver reacts with the electron donor groups to interfere with enzymatic cellular reactions. Silver also interferes with energy dependent ionic transportation and disturbs the membrane dependant ionic distribution [Semeykina 1990 and Dibrov 2002].

Being a heavy metal, silver is toxic to the human body as well. Excess of silver in the human body gives rise to a condition called Argyria, a sort of silver poisoning. Silver has been studied with respect to its effect upon many different cellular types. In 1987, Rungby examined the effects of silver ion concentration between 20 and 40 µM, using silver lactate as a test medium for cultured mouse peritoneal macrophages [Rungby 1987]. At these sustained concentration levels the macrophages demonstrated approximately 8 days survival before exhibiting cellular detachment. The cellular toxic levels of hepatocytes was determined to be both time and concentration dependant.
Toxicity was observed at 30\(\mu\)M [Baldi 1988]. These results were confirmed by Runyby when he proposed a silver concentration range of 30-70 \(\mu\)M to show appreciable cytotoxic effects within hepatocytes [Rungby 1990]. When characterizing the effects of silver for extended periods of time it was found that a 12\(\mu\)M concentration was needed to produce toxic cellular effects when looking at human monocytes. When concentrations were decreased to 8 \(\mu\)M the signs of cytotoxicity were apparent by 4 weeks [Wataha 2002]. When examining the cytotoxic effects on burn patients a 50 \(\mu\)g/ml dose was lethal to keratinocytes and a 33\(\mu\)g/ml dose was lethal for fibroblasts [Poon 2004]. The silver safety report recommends a limit to the total silver intake per day from all sources not to increase 0.005 milligrams per kilogram of the body weight [Newman 1999]. There is evidence that silver is excreted at a rate greater than that required for bactericidal effects. Total body silver excretion can be 3.97 milligrams per 24 hour cycle [Altman 1999], and the World Health Organization recommends a dietary intake of silver of 0.08 milligrams per day. Thus it can be concluded that silver can act as a safe anti bacterial if a controlled concentration is released inside the body.

Fuller’s (2005) work emphasizes that ionization enhances the inherent antimicrobial activity of certain noble metals and increases their efficacy for treating infections. The number of ions generated by passing a given current across the metallic surface is directly proportional to the amount of electrical current applied to the surface (Faraday’s Law). If properly controlled this provides a unique mechanism for the controlled evolution of ions from the surfaces of noble metals.

Creams, solutions, foils, and mixtures have been studied as mediums for the delivery of silver [Yin et al., 1999]. But the problem is that silver from these compounds and metallic surfaces such as foils does not dissociate. Silver compounds that dissociate easily, such as silver nitrate are locally sclerosing. Resistance to silver by some strains of bacteria has also been observed in the cases when silver is ionized from one of its compounds such as silver nitrate or silver sulfadiazine [Stern, 1984]. The study from Fuller also indicates that metallic silver ionized from a source of 99.99% purity silver is very effective in inhibition the growth of most of bacterial species (gram negative and
gram positive) and fungi. Though it is well established that silver ions can act as excellent anti bacterial, there is no proper delivery system for the ions to the site of infection. This results in the limited use of metalloids in a clinical setting to silver for the prophylaxis of gonococal ophthalmologic infections in newborns and in the treatment of burn patients and leg ulcers. It is essential to ensure that the ionic form is delivered to the site of infection continuously in a controlled concentration that is locally toxic to the bacterial species but remains below total body toxicity levels. As such, a control mechanism for the evolution of ions needs to be developed.

This thesis evaluates an engineered treatment system to reduce RHD associated infections based on using controlled amounts of antimicrobial metals or metal alloys in their electrically generated ionic form. The design proposed uses a silver anode and a silver cathode separated by electrical insulation. Simple electrical control is obtained by generating ions with a small electrical current running through a solid silver surface. In this scenario, under the effect of the electric potential, the ions are generated and move from cathode to anode, passing over the separation between the electrodes via a conducting media (agar, in this case). Since the microbial species are contained in the conducting media itself, essentially they become the silver ion carriers. This causes the silver ions to be in direct contact with the microbial species and hence a direct attack to the cells is achieved. The performance of this system is dependent mainly on the control of the ionic concentration of the silver ions. The factors affecting the ionic concentrations and the surface charge density were identified as the current in the system, anode-cathode separation and the surface area of the silver wire exposed. The control and optimization of these factors was done by devising a set of experiments with the following design variables:

- **Circuit polarity:** Both anodic and cathodic devices were checked for the functionality.
- **Discharge amperage:** 5 different current levels were generated by using different resistors to study the effect of current and voltage
- Anode-Cathode separation: The insulation between the anode and the cathode was varied from 6 mm to 15 mm. This is the distance over which the media containing the microbial species acts as a carrier of ions.
- Electrode Surface Area: Affected by the length of the electrode (varied from 15 mm to 6 mm) and the diameter of silver wire (two diameters 0.75 mm and 0.5 mm).

**Summary:**

This chapter has identified the cost and difficulty associated with RHD related infections. These infections cost the US about 2 billion dollars per year and involve extensive treatment to combat. The continual use of antibiotics has increased the resistant strains of bacteria, giving rise to the need to alternative antibiotic techniques. The historic use of silver as an antibiotic indicates a solution, but there is a lack of a proper system to deliver silver in its ionic form. An approach for designing a system using silver ions which could act as a promising antibacterial system has been briefly described for the controlled delivery of silver ions to the site of RHD infections.
Chapter 2
LITERATURE REVIEW

Osteomyelitis, bone infection, and different treatments which have been or can be employed to combat the infection are reviewed, followed by review of osteomyelitic infections with respect to residual hardware devices and their proper treatment. The literature related to medical use of various metal ions, especially on historic use of silver and its antibacterial properties, is discussed focusing on the reaction on bacterial cells as well as within the human body. Issues related to silver resistance in some bacterial species and other issues related to the delivery of silver ions to the site of infection are described.

Osteomyelitis

Osteomyelitis can be defined as “an acute or chronic inflammation of the bone irrespective of the cause” [Norden 1988]. It is usually characterized by progressive inflammatory destruction and erratic new bone apposition. This condition commonly occurs in vertebrae and in bones of the feet in patients with diabetes or at sites of bone penetrated during contaminated fracture and bone surgery. Osteomyelitis is categorized as two types: hematogenous and direct osteomyelitis. Hematogenous osteomyelitis is caused by bacterial seeding from the blood, and occurs primarily in children. Direct or contiguous inoculation osteomyelitis is caused by direct tissue contact with the bacteria during trauma or surgery. Various animal models have been studied to explore the pathogenesis of osteomyelitis [Norden 1988].

Osteomyelitic infectious organisms are divided into groups according to the age of individuals. The infectious organisms causing osteomyelitis include *Staphylococcus aureus* (most common), followed by *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Some fungi parasites and viruses are also known to cause bone infections, but these infective organisms are age dependent. Group B *Streptococcus* and Gram-negative
bacteria infect the newborns, while adolescents and adults tend to get infected with *Pseudomonas* and *Mycobacterium Tuberculosis* [Offiah, 2006].

It is difficult to treat osteomyelitis because of several key factors. When an infection broods, cytokines are generated locally by the inflammatory cells which are osteolytic in nature and thus open more non-vascular routes for the bacteria to grow. Another concern is the small size of the vascular channels. These microscopic channels in the bone are too small to allow the antibiotics to go through them, while the bacteria can easily pass through, grow and multiply. Toxins are also released by the bacteria when the macrophages attack them. The body’s natural response is to wall off the outside world, resulting in small vessel necrosis and ischemia, leading to eventual bone death. The most important aspect that makes osteomyelitis difficult to treat is the bone’s limited capacity to regenerate. As the osteolytic substances increase and pus spreads into vascular channels, the blood supply to the area decreases and necrotic bone prevails [Lew 1997].

These infections are also commonly associated with other risk factors. Any individual with a piece of residual hardware device such as a prosthetic device or fracture fixation devices is at a high risk of infection. These RHD related infections are becoming common. People suffering from diabetes mellitus, sickle cell disease, rheumatoid arthritis and cancer are also at high risk for developing osteomyelitic infections [Pinak and Simonis, 2002, Deacon et al. 1996].

**Osteomyelitis and Prosthetic devices**

In the case of an infected implant, it is difficult to treat these infections for several reasons. The implant surface provides an excellent surface for the bacteria to adhere onto and to multiply. Moreover, the bacteria are capable of forming a slime layer around them, called the biofilm or the glycocalyx layer, which is generally a viscous polysaccharide or polypeptide substance. The glycocalyx layer helps bacteria to resist phagocytic engulfment by the white blood cells. It also acts as a shield to the bacteria preventing antibiotics from passing through the biofilm.
*Staphylococcus aureus* expresses adhesions for some specific bone matrix components (fibronectin, laminin, collagen, and bone sialoglycoprotein), which help it to adhere to the bone. It also expresses collagen binding adhesion to adhere to cartilage. Many documented *in-vivo* clinical studies, [Vandaux 1989,1993] have shown the role of these adhesions in *S. aureus* attachment to the bone. Figure 1 shows the role of fibronectin-binding adhesions and the degree of adherence of *S. aureus* to a surgically implanted device in bone. After the pathogen has attached to the bone, it starts expressing phenotypic resistance to antimicrobial treatment which leads to high failure rates for short courses of therapy [Lew et al. 1997]. The implant hardware in the middle of the native blood supply causes a reduced blood flow to the area and makes treatment of infection even more difficult since it is the blood flow that carries the antibiotics.

![Titanium miniplates and miniscrews (left) and a radiograph obtained six weeks after the implantation of these devices in the iliac bone of a guinea pig (right).](image)

**Figure 1: Titanium miniplates and miniscrews (left) and a radiograph obtained six weeks after the implantation of these devices in the iliac bone of a guinea pig (right).** [Lew et al. 1997]

**Treatment of Osteomyelitis**

The need to develop prophylactic equipment to be used along with careful surgical procedures to avoid the infection have been emphasized [Bengtson 1993]. The treatment method for osteomyelitis depends on the type of infection, which depends on
the oxygen tension of the tissue at the infected site and the potential revascularization [Bengtson 1993]. Treatment for acute bone infection typically involves extended hospitalization, joint debridement, aggressive antimicrobial therapy, total joint removal followed by total joint replacement. If the infection spreads too far too be treated this way, sometimes amputation is done to eliminate the infection. However, to achieve total elimination of the infection, hardware removal is followed by extensive antimicrobial and joint immobilization therapy for at least six weeks. In 2004, Miller reviewed the success of this antimicrobial therapy and found that six percent of patients with infection, even after extensive antibiotic treatment, had initial amputations and three fourths of those remaining had recurrences of infection within the first six months [Miller 2004].

Drug impregnated implants are also considered as a potential option to prevent or treat these RHD infections. Potential bioimplant materials include polymethylmethacrylate (PMMA), inorganic minerals, fibrin and synthetic polymers [Wang et al 2002]. In the 1970s, Buchholz and Engelbrecht introduced antibiotic impregnated bone cement to prevent or treat the bone infections. These can provide a high concentration of the antibiotics locally through diffusion and so it is not dependent on the vascular perfusion at the infected site. In 1993, however, it was reported that gentamicin impregnated PMMA did not had any benefit over systematic antibiotics, and these impregnated material often have to be eventually removed from the body [Nelson et al, 1993].

Antibiotic Resistance

Antibiotics are not very effective against slow growing bacteria because active wall synthesis is necessary for an antibiotic to be bactericidal. *Staphylococci* have an ability to acquire a very slow metabolic rate in a phenotypic alteration referred to as a small colony variant [Ciampolini and Harding, 2000]. Due to the continued use of antibiotics, many pathogens are now expressing resistance against various antibiotics. This has caused an ever increasing concern in the medical fraternity. Forbes, (June 19 2006) reported that $30 billion is spent annually to fight infections, and that *drug*
resistant infections kill more Americans every year than AIDS and breast cancer combined.

With antibiotic resistance becoming a major issue, and the cost and trauma associated with the residual hardware related infections; there is a need to look for alternative ways to combat these infections. The controlled use of heavy metals looks promising.

**Bactericidal Silver**

Metals, particularly silver, gold and copper have been used in medicine for decades for their antimicrobial properties and have been used recently as components of wound dressings and catheter coatings. Silver is known to have the best prophylactic profile among these metals and has been historically used in a variety of ways. Silver was first reported to be a bactericidal in the eighth century [Gager 1935 and Hill 1939]. Silver has been used as a water purifier, in wound care, burn cure and recently in reconstructive orthopedic surgery, cardiac devices, catheters, bone prostheses, and surgical appliances.

Klasen has reviewed the use of silver as a treatment of chronic wounds and ulcers beginning with the early use of silver to prevent ophthalmia neonatorum, wound infections, abscesses, burns and venereal diseases. He also cites the use of silver for operative hernia and prostatectomy treatment, preparation for skin graphs, and exema treatment. However there have always been issues concerning the proper delivery mechanism for these therapies [Klasen 2000 A and B].

**Pharmacodynamics of Silver and Health Effects**

Exposure of the heavy metal in high concentrations is harmful to the human body. Experiments have been done to identify the dose of silver acetate required to kill test animals. LD$_{50}$ exposure levels of 0.22 mmol/kg in rats are reported [Horner 1983]. But
these doses will have to be doubled if used for human subjects. Study on rats and mice had indicated that the body retains from 1-10% of the amount of silver which is inhaled or ingested [East 1980 and Furchner 1968]. Following ingestion or inhalation of silver compounds, silver is complexed with transferrin in the blood and distributed throughout the body [Matuk 1981]. Silver is excreted mainly via the bile (>90%) in the feces, and in very small amount in the urine, with glutathione being the major transfer molecule [East 1980, Furchner 1968, Newton 1966 and Alexander 1981]. Recovery of silver in rats feces was 98-99% on the second day after oral exposure to silver; and it was 94% in monkeys [East 1980]. The blood, fecal and urine concentrations of silver increase due to an increased industrial or medical exposure to silver [DiVincenzo 1985, Jensen 1988, Wan 1991].

Toxic silver levels for hepatocytes were determined to be both time and concentration dependant, but could be detected at 30µM [Baldi 1988, Rungby 1990]. Wataha determined that a 12 µM concentration was needed to produce toxic effects in human nonocytes when used over an extended period of time (4 weeks) [Wataha 2002]. The cytotoxicity was determined at the dose of 33 µg/ml in burn patients [Poon 2004]. Silver toxicity has been reported to occur at serum silver levels as low as 0.3 µg/mL and manifests as argyria, leucopenia and alterations in renal, hepatic and neural tissues [Tweden 1997 and Wan 1991]. Silver impregnated bone cement in high concentrations has limited the growth of bacteria, but at the same time a patient developed muscular paralysis, as a result of high ionic concentration of silver (103.2 µg/L) from silver impregnated bone cement [Sudmann 1994].

Human hypersensitivity (allergic reactions) to certain heavy metals has been documented. However, there has not been any severe allergic reaction to silver [ASTDR 1990]. Allergic contact dermatitis associated with silver has been reported in the form of a delayed T cell mediated response. In case of allergy, use of topical steroids along with removal of the source of contact can be effective.
Chronic silver accumulation results in a condition which is called argyria, a case of silver poisoning. Ingestion of soluble silver salts between the ranges of 1-30 g have been known to cause argyria [Nordberg 1988]. Antismoking lozenges containing silver acetate, breath mints coated with silver, silver nitrate solutions for the treatment of gum disease, and silver nitrate capsules for relief of gastrointestinal discomfort are identified as main ingestion sources of silver which cause argyria [ATSDR 1990 and Stokinger 1981]. This condition is characterized by an irreversible gray or blue-gray discoloration of the skin and mucous membranes. Silver metabolism is modulated by induction and binding to metallothioneins. This complex mitigates the cellular toxicity of silver and contributes to tissue repair. Blond individuals and the skin exposed to sunlight is more susceptible to argyria than others [Nordberg 1988]. Silver-containing granules are seen concentrated in basement membranes and elastic fibers surrounding sweat glands, during histopathologic examination of the skin of these individuals [ATSDR 1990].

Silver Ions

The use of silver in medical applications has evolved over time. In 1997, a wound dressing called Acticoat, was produced with a 0.2 to 0.3 mg of silver coating per mg of high density polyethylene. With a prolonged release of silver in an aqueous environment at local concentrations of 50 mg/L to 100mg/L for 48 hours, Acticoat showed minimal toxicity to the mammalian tissue [Burrell 1999]. Some other fabrics using silver coated nylon have also been developed as antibacterial fabrics [MacKeen 1987 and Deitch 1983]. Catheters coated with silver and silver hydrogel were also tested clinically, resulting in a minimal percentage reduction in the growth of colonized bacteria [Johnson 1990]. Catheters coated both internally and externally were also minimally effective in reducing colonization (9% for coated vs. 10% for non-coated [Riley 1995]. The colonization rates were reduced by 30% when a silver hydrogel coating was used as it allowed for a greater release of silver ions, [Maki 1998]. In a study by Trerotola it was demonstrated that there was no significant reduction in rates of catheter colonization (0.28 vs. 0.13 cases per 100 catheter-days) and catheter-related infection (0.18 vs. 0.11 cases per 100 catheter-days) in patients receiving silver-coated versus uncoated catheters.
Other studies of silver coating included peritoneal catheters with no significant differences in the rates of exit site infections (24% vs. 16%), sinus tract or tunnel infections (12% vs. 12%) and peritonitis (16% vs. 18%). Also silver coated stainless steel external fixation pins demonstrated similar rates of infection to regular pins (83% vs. 92%) [Pommer 1998]. In some cases silver coated prosthetic heart rings proved to be effective against the bacterial growth in vitro, but they were harmful to the myocardial cells [Nelson 1999]. Some compounds of silver which have proved to be bactericidal for twenty two bacterial species include silver sulfadiazine, at a minimal concentration of 12.5 µg/ml [Carr 1973].

Silver’s antibacterial action has been further examined when combined with small amounts of direct current. A breakthrough in vitro result was obtained when silver was used in conjunction with low electrical currents (20-75 µA). The electrical charge stimulated liberation of silver ions from the surface of the catheter, thereby producing zones of inhibition against potential pathogens [Raad 1996].

**Silver Ion Generation**

Early experiments using current with silver to generate silver ions in an *in-vitro* environment demonstrated that 2.0 to 20 µA/cm² of positive direct current per square centimeter on silver electrodes had a bacteriostatic effect [Rungby 1987 and Barranco 1974]. The use of silver electrodes to treat infected bone fractures have also been documented [Becker 1978]. It was also reported that nearly 100% of approximately 3000 colonies of *Staphylococcus aureus* were killed using a 12 µA DC current with silver electrodes. It was suggested that the small free silver ions were able to penetrate any structure with an aqueous component [Colmono 1979]. An elaborate experiment was conducted by Hall using fourteen strains of bacteria. A low intensity direct current generator was connected to a pure silver electrode and it was found that all of the fourteen strains had some growth inhibition at a minimum ionic concentration of 45 parts per million [Hall 1987]. Thus it was concluded that silver ions can be used as potential bactericidal agents. Quantitative results show that the concentration of silver needed to affect bacteria was well below the concentrations required to produce human cellular
toxicity. The effect of silver ions on mammalian cells was evaluated by Berger et al. They found that a current of 75 µA did not affect mammalian cells in a culture [Berger 1976]. A silver iontophoretic vascular catheter utilizing up to 75 µA current was tested in humans with no adverse effects [Raad 1996], which corresponds to the British Standard for Medical Equipment which states that currents in this range are unlikely to cause any adverse events in humans [British Standard 1989].

When testing activated silver coated fibers MacKeen demonstrated that a silver ion concentration of 1.24 µg/ml resulted in a tenfold decrease in bacterial growth within 147 minutes. Increasing the silver concentration to 1.93 µg/ml resulted in a tenfold bacterial reduction within 13 minutes of exposure [MacKeen 1987]. Silver ions also proved to be effective against all bacterial strains when tested clinically in treatment of orthopedic infections with negligible effect on human tissue [Becker 1978].

In general, the bactericidal effect of silver is not fully understood. It is believed to attack the bacterial cell by interfering with the respiration mechanism and forming complexes with the bacterial cell walls, membranes, enzymes and nucleic acids [Slawson et al. 1992]. It has been established that silver reacts with electron donor groups to interfere with enzymatic reactions at the cellular level, also affecting the energy dependent ion transportation to disturb the membrane dependant ionic distribution [Semeykina 1990 and Dibrov 2002]. Research has also demonstrated the different ways in which silver interacts with the micro organisms as shown in Figure 2 [Russell et al. 1994]. The sites shown on Figure 2 are mostly proteinaceous in nature and their alterations would affect the cell structure and metabolism, thus causing cell disruption. A close mechanistic study on the effect of silver on *S. aureus* and *E. coli* was done in 2000 [Feng et al. 2000]. They cultured both organisms with AgNO₃ and the resultant biomass was then subjected to transmission electron microscopy (TEM). The use of silver for the treatment of periodontitis was introduced by developing a delivery system [Straub et al. 2001]. Their method used a local delivery device that delivered silver to the site of infection from a 12% silver nitrate source. Their study suggested that silver nitrate is
effective against gram negative bacteria, but there has been no reporting on the effect of silver ions on gram positive bacteria.

Figure 2: Interactions of silver with micro-organisms. [Russell et al. 1994]

In addition to its bactericidal activities, researchers have found that silver is also fungicidal and virucidal. Activated silver has also been found to be antifungal at concentration less than 4.7 µg/ml for all species tested [Berger 1976]. Its molecular mechanisms of fungicidal action are similar to those observed against bacteria with protein damage through sulfhydryl group interactions. Its virucidal mechanisms of action involve alteration of viral protein, but also suggest damage to the viral nucleic acid [Maillard 2001].

Results have demonstrated effective activity both from silver ion-beam-implanted surfaces and polymer coating with metallic silver. However, several clinical studies have shown a lack of efficacy with silver impregnated devices. This suggests that silver-
coatings have no detectable antimicrobial effect if there is no silver ion release [Wright 1978, Burrell 1999, Tredget 1998].

It has been established that silver is most effective in its ionic form. In a study conducted by Fuller, silver with DC current as low as 20 µA was found to be bacteriostatic [Fuller 2005]. He developed a system in which silver ions were continuously generated on agar plate inoculated with bacteria. He designed a nested experiment with eight high purity metals (silver, copper, titanium, gold, cadmium, nickel, zinc and stainless steel AISI 316L) and their electrically generated ionic forms against 5 bacterial species and one fungus commonly associated with osteomyelitis. Visible inhibition zones were formed around the silver electrode demonstrating a bacteriostatic effect for five different bacterial species. Based on his findings, he proposed a design for a prosthetic using electrically activated silver coating.

Silver resistance

Bio-resistance to silver has been observed in cases when silver is ionized from one of its compounds like silver nitrate or silver sulfadiazine. Bacterial resistance to heavy metal ions can result from energy-dependent ion efflux systems rather than chemical detoxification. It has been suggested that bacterial resistance can occur both due to an efflux pump or a binding protein produced by the bacteria [Gupta 1998]. Literature suggests that the mechanism of resistance for heavy metals is associated with the energy-dependent ion efflux systems. In Gram-negative bacteria, biocides are blocked from reaching targets in the cell by the outer membrane (OM) and active efflux mechanisms [Silver and Phung 1996]. Efflux pumps are composed of proteins either as an ATPase or chemi-osmotic cation/proton antiporter. Ag⁺ has been associated with both of these mechanisms [Gupta and Matsui 1999]. A reported resistance of bacteria to silver within drinking water containment units was found to be due to a sustained low concentration of silver ions which was not bactericidal [Mietzner 1997]. The proposed minimum bactericidal concentration for silver ions in water is 20-40 µg/L [Spadaro 1974, Liu 1998, and Stout 1998].
Due to the widespread use of silver in wound care and dental care, it is probable that silver resistant strains of microbes may develop. An important aspect to be considered while working with silver is to identify if there has been any kind of silver resistance reported in the bacteria. There have been evidence of resistance in several cases in the late 1970s by gram-negative isolates *P. aeruginosa* [Bridges et al. 1979] and *Salmonella typhimurium* [McHugh et al. 1975]. Researchers have also reported an isolate of Salmonella that exhibited a plasmid mediated silver resistance. The plasmid system contains a small periplasmic silver binding protein SilE, that binds silver ions specifically at the cell surface and protects the cell from toxicity [Gupta and Silver 2000].

There has been no concrete explanation for a mechanism of silver resistance in gram positive bacteria; however, it is believed to be on the same basis as the cadmium and copper resistance in bacterial cells [Silver 1996]. The resistance to cadmium is due to genes CadA and CadB, while the proton pump is responsible for copper resistance. The suggested mechanisms causing silver resistance are:

- decreasing intracellular accumulation
- increasing the production of neutralising compounds
- reduction of the metal ions to a less toxic oxidation state.

Silver-susceptible clinical strains of *Escherichia coli* which did not contain any plasmids have also shown complete cross-resistance against silver nitrate and silver sulfadiazine. [McHugh et al. 1975]. There can be an explanation for the bacterial resistance against silver on the basis of a proposed molecular and genetic theory [Gupta and Matsui 1999]. It has also been observed that bacteria containing the silver resistant plasmids accumulate less Ag$^+$ than other susceptible strains. The presence of albumin and halide ions are expected to result in the formation of AgCl crystals, with increased adsorption causing resistance against silver ions activity. Other experiments have revealed that halide concentrations have a great impact on the sensitivity of *E.coli* to Ag$^+$ [Gupta et al. 1998].
Summary:

Silver has been used in medical care as an anti-infection agent for hundreds of years. The reasons are silver’s low toxicity in the human body and the minimal risk due to clinical exposure by inhalation, ingestion, dermal application or through urological or haematogenous routes. Silver by itself has not provided any promising results when tested in-vitro, but it has been established that silver ions have bactericidal properties. To have adequate non-toxic, bactericidal effect, silver ions need to be delivered to the site of infection at concentrations between 1.24 µg/mL and 30 µg/mL. Sudmann (1994) also concluded that the ability to release silver ions from bone cement impregnated with silver determines the bactericidal effect of silver. Also the concentration at which silver ions are toxic to humans is at least ten folds the bacteriostatic concentration. Silver ions have proved to inhibit the growth of various bacterial and fungal species in vitro, there has been no in vivo efficacy as yet demonstrated. Silver ions delivered in the form of bone cement impregnation, anti-infective creams or coatings have not been successful in providing a continual release of ions and thus not being effective against the growth of bacteria in the long term. This can be attributed the limited delivery options which can generate silver ions continuously at the site of infection and deliver an adequate concentration of silver ions to the site of infection. This emphasizes the need to develop an effective system using electrically activated silver, which can guarantee a continual and controlled release of silver ions to the site of infection.
Chapter 3
WORKING PRINCIPLE AND LIMITING CONSTRAINTS

This chapter evaluates a concept design for a prophylactic system using silver ions as the antibacterial agent [Patent Serial No. PCT/US2006/026000]. This design can be incorporated in indwelling hardware devices. The use of silver as a potent antibacterial is characterized with respect to total human life exposure, cellular toxicity and the amount of direct current required for the ionization of silver. These design constraints are discussed in general so that they are applicable to a variety of antibacterial systems incorporating silver ions. The variables affecting the system performance have been identified.

Working of the system

The proposed prophylactic system delivers silver ions as an antibacterial agent. The silver ions when delivered in appropriate concentrations to the site of infection can inhibit the bacterial growth and thus prevent the infection from spreading. One of the important concerns for the proper functioning of such a system is that the silver ions must be properly delivered to the site of infection. While various chemical reactions and physical stimulus methods can be employed to ionize silver, using electric current for controlled generation of ions is both viable and practically feasible for an indwelling system. Hence a system using electric current as the source of ionization is evaluated. It is critical that the media containing the bacteria should serve as the conductive path for the silver ions. This can be achieved if the silver ions select a path through the soft tissue infected with bacteria when travelling from anode to cathode. This implies that there must be a separation between the anode and the cathode, with a current path through the soft tissue, which acts as the conductive media. This approach would treat osteomyelitic infections which have been observed to spread to the soft tissue near residual hardware devices.
Figure 3 shows a schematic of a prophylactic system using electrically ionized silver. When this system is placed in a bacteria rich environment, the ions move from cathode to the anode due to the effect of the applied voltage through the conducting soft tissue which is infected by the bacteria. This allows the silver ions to be in direct contact with the bacteria rich soft tissue.

If such a system is incorporated as part of an indwelling hardware device, the continuous generation of silver ions would result in an inhibition of the bacterial growth, and hence infection could be prevented. Devices based on such a system could potentially replace or partner with treatment methods using traditional antibiotics.

**Design Constraints:**

Since this system would be used inside the human body, there are limiting constraints on the design that must be considered. Silver ions have been shown to be antibacterial at a local concentration as low as 1.24 micrograms / milliliter. In order to stay below the toxicity threshold limit values for metallic silver inside human body, as given by the American Conference of Governmental Industrial Hygienists [Drake and Hazelwood, 2005], the local silver ion concentration should not surpass the value of 30 micrograms/milliliter. Another important factor to be considered is that silver ions travel through the soft tissue by the mechanism of diffusion [Bong et al., 2001]. Considering these effectiveness constraints and a safety factor of three, the safe design value for local ionic concentration would be 10 micrograms / milliliter.
Since RHDs are expected to stay inside the human body indefinitely, the lifetime exposure of silver should also be taken into consideration. Based on the assumption of a maximum device lifetime of 70 years, the total silver exposure should not exceed 8.95 grams [U.S. EPA 1997].

Equation 1 gives a sample amperage calculation for a battery of 1.55 V incorporated with a resistor [Nilsson 2007]. The calculated current values obtained from using the proposed system design with resistors in series with the anode of silver wire. Sample calculation in Equation 1 is showing the total electrical current generated using a 1.5 M ohm resistor.

$$I = \frac{V}{R} = \frac{1.55V}{1.5 \times 10^6 \text{ ohms}} = 1.03 \times 10^{-6} \text{ amperes}$$  

(Equation #1)

**Equation 1**: Sample calculation showing the total electrical current generated using resistors. This calculation used a 1.55 volt battery and 1.5 MΩ resistor placed in series with the metallic wire.

Since ionization is required and we are using electric current to ionize silver ion, it is important to analyze the amount of current which can be used in the system. The first constraint is the amount of current which can be adopted for indwelling hardware devices. Weiss suggests that electric current of the order of 20 micro-amperes /square centimeter of conductive surface is safe for a human body, while current of the order of 1666.66 micro-amperes /square centimeter of conductive surface is the toxic level. Also, electric current of the order of 10 – 75 µA are believed to stimulate healing for both fracture and wounds [Weiss et al., 1980]. Currents of this order (10-75 µA) can ionize silver anodes and produce silver ions.

The amount of silver ions, released during electrical activation, are governed by Faraday’s law, as given is in Equation 2 [Fuller 2005]. This calculation is performed for a silver wire using the value of 107.868 gram Ag / 1 Faraday [De Laeter et al. 1992].
This implies that if the power source is capable of producing 1 micro ampere of current, 4.02 µg of silver ions will be produced in an hour. For the optimal working of the above described prophylactic system, its performance metric and the parameter space of different variables affecting it need to be explored in more detail.

**Performance Measure of the system:**

The prophylactic system under study is expected to continuously generate silver ions in order to inhibit the growth of bacteria. The system performance measures should relate to the effect of silver ions generated on the bacterial growth. The ability of the system to kill or inhibit the growth of the bacteria would define the antibacterial potential of the system. This ability to inhibit or kill the bacteria can be quantified in a number of ways. Traditionally two different methods are used to check the efficacy of an antibiotic (silver ions in this case). In first method, antibiotic is released in bacteria rich media with a known concentration, the concentration count of the bacteria is performed and reduction in bacterial count as a function of elapsed time is determined. Another method is to place the antibiotic on a media (which simulates the behavior of human tissue) inoculated with bacteria in a Petri dish. If the bacteria are susceptible to the antibiotic, they would not be able to grow around the system and an area of clearing would be visible. This area is called a zone of inhibition, and measuring the zone of inhibition is a good metric to evaluate the performance of the antibiotic [Boyd and Hoerl, 1981].

This prophylactic system is expected to work in contact with human soft tissue, and the agar media very closely resembles the properties and behavior of human soft tissue. It is for this reason that this second technique has been chosen here to measure the efficiency of the system. The width of the bacterial inhibition zone is considered the response variable or the performance measure of this system.
Parameters affecting Performance:

Several parameters affect the performance metric (the inhibition zone width) of silver ion release systems. The following are considered important variables which control the effective release of silver ions in the system, and hence the response variable, which is the zone of inhibition. These parameters include:

**Silver Ion Concentration:** Since silver ions inhibit bacterial growth, the concentration of silver ions in the bacterial environment is an important parameter affecting the size of the inhibition zone. More the concentration of silver ions, the inhibition zone is expected to be larger. Under the constraints of silver ion concentration in the human body as discussed above, we need to identify the maximum and minimum silver ion concentrations required for an effective kill.

**Polarity of the system:** Different concentration gradients of silver ions are expected around the anode, the cathode and through the separation zone across the two electrodes. This gradient would affect the distribution of inhibition zone around the system. Hence, the polarity of the system would influence the inhibition zone.

**Separation between anode and cathode:** For the ions to move through the bacteria containing media, the separation between the anode and cathode becomes critical. An increase in the separation would enable the ions to move through a larger portion of the bacteria rich media and hence a larger inhibition zone. However, more separation also increases the resistance between the anode and cathode changing the effective current travelling through the system.

**Amperage of the system:** An increase in the system resistance due to increased electrode separation would require an increase in voltage applied by the battery. However, there are constraints as discussed above for the amount of ionization current which can be used.
**Surface Area of anode and cathode:** The silver ion concentration is also a function of the surface area of the anode and cathode, making the length and diameter of anode and cathode as important system parameters to be evaluated.

These independent variables are studied with respect to their limiting values and effect on the inhibition zone produced by the system in the form of a mathematical model as described in the following section. This mathematical model would be useful in quantifying the parameter space of these variables.

**Mathematical model for system Performance:**

In the mathematical model, the response variable is the zone of inhibition and the predictor variables are the circuit polarity, voltage and amperage of the system, the surface area of the anode, surface area of the cathode and the separation between anode and cathode. The last three variables are the geometrical system variables and are shown schematically in Figure 4.

![Figure 4: Schematic describing the geometric parameters of the device](image)

The response variable, the width of the bacterial inhibition zone area can be thus written as

\[ Z = F (A_1, L_2, A_3, \text{Amp, Polarity}) \]

Where \( L_2=\text{length of separation between anode and cathode} \), \( A_1(\text{surface area of anode}) = \pi D L_1 \) and \( A_3(\text{surface area of cathode}) = \pi D L_3 \). The kill area is calculated by approximating the inhibition zone as a rectangle.
Summary

This chapter presented the concept of a working antibacterial system using electrically ionized silver ions. It examined the issues related to silver toxicity with respect to total human life exposure and cellular toxicity. It also identified the direct current required for ionization of silver and the safe limits of current which can be used inside the human body. This chapter also identified the independent variables important in the design of such a silver ion delivery system, and the effect of these variables on the system performance.
Chapter 4
Materials and Methodology

This chapter describes the materials used for the testing and presents the methodology incorporated to conduct experiments. A system design is proposed to evaluate the effect of control variables discussed previously on the performance of the silver ion delivery system. An experimental design is introduced to estimate the optimized ranges for these control variables.

System Design

Using the working principle as explained in the previous chapter, a device has been designed. The design enables variations in the variables which affect the performance of the system. Figure 5 gives a schematic of the device.

![Figure 5: Schematic of the proposed device design (anodic device)](image)

This represents a prophylactic system and was used for the in-vitro study with the agar media.

Experimental Design

To identify useful values for the control variables which affect the working of this system, experiments were run using the following independent variables:
• Circuit polarity: Both anodic and cathodic devices were checked for the functionality and performance in inhibition of microbial growth.
• Device amperage: Five different currents were studied.
• Anode-Cathode separation: varied from 6mm to 30 mm.
• Surface area of Anode: Both and length and diameter were varied. Length varied from 6mm to 15 mm and two diameters considered; 0.75 mm and 0.5 mm.

Functionality was checked using both the anodic and cathodic devices. Since anodic devices performed better, the experiment was designed as a fully balanced experiment with anodic devices. The devices had four anode-cathode separations (varied from 6mm to 30 mm) and three different anode lengths (varied from 6mm to 15 mm). All these devices were replicated with five different currents to study the effect of current and voltage. The current generated by each of the four different circuit resistors [10 MΩ, 1 MΩ, 100 kΩ, and 75 kΩ] was 0.15 µA, 1.5 µA, 15 µA, and 20 µA respectively, when combined into the circuit. Current across device without resistor was 1.5 A. The resistance of the agar media was estimated to be approximately 250 KΩ. Therefore, the current values achieved inside the actual system were less than these values. These parameters are tabulated in Table 1.

**Table 1: Range of experimental variables studied**

<table>
<thead>
<tr>
<th>Separation (mm)</th>
<th>External Resistor</th>
<th>Closed Circuit Current</th>
<th>Anode length</th>
<th>Anode Dia</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>10 MΩ</td>
<td>0.15 µA</td>
<td>6</td>
<td>0.75mm</td>
</tr>
<tr>
<td>10</td>
<td>1 MΩ</td>
<td>1.5 µA</td>
<td>10</td>
<td>0.5 mm</td>
</tr>
<tr>
<td>15</td>
<td>100 KΩ</td>
<td>15 µA</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>75 KΩ, 1 Ω</td>
<td>20 µA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The experimental philosophy is similar to the experiments done by Fuller (2005). The device used to study the effect of these parameters is shown in Figure 6. In this design the anode and cathode were integrated on the same wire and were separated by a small insulation. Metallic silver wire (99.97% purity) with 0.75 mm diameter and Teflon
insulated silver wire (99.97% purity) with 0.5 mm diameter (Advent Research Materials Ltd.), served as ion sources. Silver paint (ASI) with a purity of 99% was used as another source of silver. The device was made by making the anode (or cathode) with the silver wire and using the silver painted insulated wire as the corresponding cathode (or anode). A 0.75 mm diameter silver wire (99.97 % purity) and 0.5 mm insulated gap (L2) and silver paint were used to construct the device. The cathode was made by plating the insulated wire with silver paint and the insulation was stripped from the silver wire to expose the anode.

![Diagram of anodic device design](image)

**Figure 6: Anodic device design as used for the testing**

This device configuration allowed an interchange of anode and cathode. Thus we had two designs, anodic and cathodic. Length of the flexible insulated wire leading to the battery was limited from 60-80 mm.

**Materials and Methodology**

The pathogen used for this study was *Staphylococcus aureus* (ATCC number 29213) obtained from The Pennsylvania State University Animal Diagnostic Laboratory. A consistent methodology to prepare and grow the culture was adopted from the Kirby Bauer agar gel diffusion technique based on methods developed by Lucke (2002). *S. aureus* was grown overnight in 9 ml tryptic soy broth (TSB) (caseinpepton–soybean flour–peptone–solution; Oxoid Ltd., Basingstoke, Hampshire, UK). These tubes were then incubated for 3 hours at 37 °C in order to obtain log-phase bacteria growth. Under spectrophotometric control the bacterial sediment was added to clean TSB until a McFarland standard of 0.5 M was obtained. Colony-forming units (CFU) per ml were confirmed by plate counts with the use of a spiral-plater (Spiral System Inc., Cincinnati,
OH). The concentration was adjusted to fit into the desired range \((10^3 - 10^4 \text{ cfu/ml})\) using McFarland Standards. Bacteria counts were confirmed by plate counts.

These cultures were inoculated onto Mueller-Hinton agar plates [Remel, Lenexa, KS]. The agar plates were modified slightly prior to bacterial inoculation [Fuller 2005]. A small hole was burnt through the bottom side of the plate to pursue a small opening for the wire insertion as shown in Figure 7.

![Diagram of Petri dish with small hole](image)

**Figure 7: Schematic showing proper placement of small holes in the Petri dishes [Fuller 2005]**

After the wire was inserted in the agar, the media was inoculated with bacterial culture grown to the desired concentration. The surface area exposed to the agar media with the bacteria could be calculated according to equation 3 [Fuller 2005].

\[
\text{Surface Area} = \pi r^2 + 2\pi rh
\]  

(Equation #3)

Each 32mm of agar contact length provides for 1 square centimeter of contact surface area, as calculated in the surface area calculation. The wire surface area forming the cathode that is exposed to the agar was similarly estimated. The exposed surface area determines the surface charge density, which was found out to be an important device design variable.
The device shown in Figure 6 was threaded into the agar plate as shown in Figure 8. The silver device was threaded through the agar plate so that both the anode and cathode were embedded inside the agar media. The arrangement of the device embedded in agar media is shown in Figure 8.

Figure 8: Schematic of a set up incorporating anodic device, battery and external resistor with metal wires.

The resistor, the negative lead of the standard 1.55 volt battery holder and the anode (insulated silver wire), were assembled in series with a Petri dish containing Mueller-Hinton agar and the desired bacteria. The resistors were axial type metal film series with a tolerance of +/- 1%, power rated for 0.6 W, with a temperature coefficient of +/- 50 PPM. The proper resistor was soldered distally to the 70 mm length of wire and proximally to the positive lead of a standard 1.55 AA battery holder.

Once integrated, the agar plates were then inoculated with the bacteria and were incubated in air at 37° C for 24 hours and examined for bacterial growth or zones of inhibition. Control plates were run in each metal trial for each experiment. In the control plates, metallic silver wires were embedded as described but they were not electrically powered and hence produced no ionization. After a bacteria contact period of 24 hours, the inhibition zone was measure and data was recorded for each experimental alternative.
Summary:

This chapter presented the experimental design for the evaluation of the independent variables which affect the performance of the silver ion system. The device designed to test these parameters was also explained. This was followed by a discussion of the materials required for the experimentation and the methodology incorporated.
Chapter 5
Results and Statistical Analysis

This chapter presents the results of the experiments performed to determine the factors that influence the performance of the system using electrically ionized silver as an antibacterial agent. The control parameters of current, polarity, anode/cathode surface area and the separation between anode and cathode were varied for different devices and their effects on the width of the inhibition zone was measured. It was a fully balanced design of experiment using anodic devices.

1. Effect of Circuit Polarity:

The concentration gradient of silver ions between the anode and cathode would affect the distribution of inhibition zone around the system. Hence, an experiment was performed to test the effect of polarity of the system on the inhibition zone.

Two separate devices were used for this study:
1. Anodic: the silver wire was connected to the (+) terminal of the battery (anode)
2. Cathodic: the silver wire was connected to the (-) terminal of the battery (cathode)

Both the devices had same surface area, anode-cathode separation (6 mm) and applied voltage values (1.5 A). These devices were then placed in the agar media inoculated with bacteria. It was found that both the anodic and cathodic devices provide good inhibition against the bacterial growth as shown in Figure 9. With both systems, a wider inhibition zone was evident at the anode along with a smaller inhibition zone at the cathode in all the cases.
Figure 9: Zone clearing for anodic and cathodic devices with 6 mm electrode separation, no external resistor, electrode lengths of 10mm each

Figure 9 shows images of the cultured plates with zones of inhibition for both anodic and cathodic devices in the agar media. The inhibition zone is more evident and larger around the anode in both the cases. Hence it is concluded that the anodic end is more effective in producing an anti bacterial environment.

One of the possible reasons that the inhibition width is larger around the anode is that the anode has a higher concentration of silver ions around it. Silver is ionized at the anode and moves across to the cathode as electrical current flows through the device. However, the diffusion of silver ions through the separating media is limited which is why the local concentration of silver ions is lower near the cathode.

2. Effect of Amperage:

Since the ionization of silver in the system depends on the circuit current, it is important to study the effect of current on the proposed system. The discharge voltage was kept constant throughout the study as 1.5 V. Amperage was varied by using different resistors in the devices. Devices with different amperage values were inserted in the agar plates showed clearance at the anode. The length of the anode was held constant at 10 mm while the length of both the separation and the cathode were also kept constant at 6 mm. This test was done with all anodic devices. All other variables such as anode surface area, cathode surface area and the separation between anode and cathode were kept
constant. So the only predictor variable for the inhibition zone is device current. The areas of zones of inhibition are tabulated in Table 2.

**Table 2: The zones of inhibition using different silver ionization currents for anodic device**

<table>
<thead>
<tr>
<th>External Resistor</th>
<th>Device Current</th>
<th>Zone width</th>
<th>Zone Area (mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ω</td>
<td>1.5 A</td>
<td>11 mm</td>
<td>110</td>
</tr>
<tr>
<td>75 KΩ</td>
<td>20 uA</td>
<td>7 mm</td>
<td>70</td>
</tr>
<tr>
<td>100 KΩ</td>
<td>15 uA</td>
<td>6 mm</td>
<td>60</td>
</tr>
<tr>
<td>1 MΩ</td>
<td>1.5 uA</td>
<td>4 mm</td>
<td>40</td>
</tr>
<tr>
<td>10 MΩ</td>
<td>0.15 uA</td>
<td>2 mm</td>
<td>20</td>
</tr>
</tbody>
</table>

The results from Table 2 indicate that lower values of current generated smaller inhibition zone widths. This can be attributed to the reduction in ionic concentration of the silver ions produced with the decreasing amperage.

![Figure 10](image1.png)

**Fig 10:** The effect of current on microbial inhibition zone area. This picture gives the inhibition zone for anodic devices with 6 mm electrode separation, 31.4 mm$^2$ anode area and device currents of a. 1.5 A device, b. 20 µA and c. 15 µA

Figure 10 shows the images of inhibition zone observed on the cultured plates with different current values, the lowest of them being 15 µA. The inhibition zone area decreases noticeably when the current value was changed from 1.5 A to 20 µA, but there is a little difference in inhibition zone area between the 20 µA and 15 µA devices. The inhibition zone area decreased further at current values of 1.5 µA and 0.15 µA. Hence, the voltage and current values appear to be the important factors affecting the inhibition
zone area. The device with 20 MΩ resistance (current = 0.075 µA) did not generate a visible inhibition zone, though there was some clearing. Since the agar media itself has some resistance (on the order of 250 KΩ across a 50 mm agar distance), a current value of 0.15 µA obtained with a 10 MΩ resistance is considered as the minimum required current for the device to work effectively.

The results from Table 2 indicate that the inhibition zone width increases with the increase in circuit current. This can be attributed to the increase in the concentration of silver ions produced with increasing amperage. It should be noted that the inhibition zone area follows a linear relationship with log(current) as shown in Figure 11.

![Area of zone of Inhibition Vs log(Current)](image)

![Figure 11: Relationship between the inhibition zone area and log(current). The anodic device with the 1.5 A current had a maximum width of inhibition zone, 20 uA and 15 uA anodic devices do not have much difference and anodic device with a 0.15 µA current gave the minimum inhibition zone area.](image)
3. Anode-Cathode separation

The separation between the anode and cathode is the region where silver ions travel through the media via the microbial species. This region has a continuous supply of silver ions from the device into the media. The effect of varying the separation between the anode and the cathode on the inhibition zone was measured. Four different separations of 30mm, 15 mm, 10 mm, and 6 mm between the anode and cathode were tested in the devices. The length of anode and cathode were kept constant at 10 mm and 6 mm respectively. The devices with 15mm, 10mm and 6 m separation had an external resistor in the circuit of 75 KΩ. Since the agar medium has resistance of its own (250 KΩ across length of 50 mm), the overall resistance of the device varies as the length of separation between the anode and cathode is changed. In order to estimate how the resistance of the media between the electrodes varied with the separation between them, the resistance of the agar media was measured across different separation points. The measured values of resistance were plotted against the separation (in mm), and a linear increasing trend was observed. This relationship is shown in the graph in Figure 12. The external resistor adds up in series with the resistance due to the separation between the electrodes. As a result, the total resistance of the device increases with increasing separation between the electrodes.
Figure 12: Change in resistance with BHI agar distance measured using anodic devices with 10 mm electrode lengths, an external resistor and five different electrode separations

The resistance follows a linear trend with increasing separation between the anode and the cathode. The devices with different separations were used to measure the effect of separation on the inhibition zone area. Of these, three devices had an external 75 KΩ resistor. The effect of the added resistance in series on the zone of inhibition is given below in Table 3.

Table 3: Total resistance in the circuit and its effect on the zone of inhibition

<table>
<thead>
<tr>
<th>Separation (mm)</th>
<th>Resistance due to agar (KΩ)</th>
<th>External Resistor (KΩ)</th>
<th>Net circuit Resistance (KΩ)</th>
<th>Net current (µA)</th>
<th>Zone width</th>
<th>Zone Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>148</td>
<td>0</td>
<td>148</td>
<td>10.13</td>
<td>4 mm</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>75</td>
<td>145</td>
<td>10.34</td>
<td>4 mm</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>75</td>
<td>130</td>
<td>11.53</td>
<td>5mm</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>75</td>
<td>115</td>
<td>13.04</td>
<td>5mm</td>
<td>50</td>
</tr>
</tbody>
</table>
Increasing the separation between the electrodes decreased the net current in the circuit (Ohm’s Law). It is proposed that the resistance due to separation can be adjusted with an external resistor in the devices. The fit equation obtained from the graph shown in Figure 12 is of the form $y = Kx + C$, where $y$ represents the resistance due to separation, $K$ and $C$ are constants while $x$ is the separation between electrodes. When this additional resistance due to separation is connected in series with an external resistor $R_0$, the net resistance ($NR$) of the circuit becomes,

$$NR = R_0 + Kx + C \quad \cdots (4)$$

Using eq. 4, the separation $x$ required to replace $R_0$ is given as $x = \frac{NR - C}{K}$.

To validate this hypothesis, an experiment was performed with two devices. The first device had an electrode separation of 30 mm and no external resistance. The second device had an electrode separation of 15 mm and an external resistor of 75 KΩ. The area of zone of inhibition was same in both the cases, as shown in Figure 13.

![Figure 13: Inhibition zones produced by using anodic devices with a) separation of 30 mm (=148 KΩ) and, b) separation of 15 mm (=70 KΩ) + 75 KΩ external resistor](image-url)
From the above analysis it can be observed both the external resistance and the resistance due to the electrode separation determine the net resistance of the system. The net current is dependent on the net resistance. For future analyses we have used net circuit current as an independent variable.

**Surface Area of the anode**

The surface area of the anode and the cathode is an important factor in determining the surface charge density. This parameter controls the concentration of silver ions generated which in turn produce inhibition against microbial growth. Two dimensions that determine the surface area (and hence the surface charge density) are the length and the diameter of the silver wire. Changing either of them results in altering the surface area. However, in this study, the diameter of the wire was kept constant and only the length of the anode and cathode wires was varied. In additional tests the diameter was varied keeping the length constant for the anode and cathode.

**Effect of Changing Length:** There was no significant effect upon changing the length (and hence the surface area) of the cathode from 6 to 15 mm. The diameter of the wire was kept constant at 0.5 mm, separation between anode and cathode was fixed at 6mm while cathode length of 6 mm and current value of 20 μA was used. The length of anode was varied from 15 mm to 6 mm. Table 4 summarizes the inhibition zone area observed for different anodic surface area resulting from different anodic lengths.

**Table 4: Effect of anode surface area on zone of inhibition with anodic devices of 20 μA device current, 6mm electrode separation, 6mm cathode length and 0.5 mm wire diameter**

<table>
<thead>
<tr>
<th>Anode Length (mm)</th>
<th>Anode Surface Area (mm²)</th>
<th>Inhibition Zone Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>47.1</td>
<td>105</td>
</tr>
<tr>
<td>10</td>
<td>31.4</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>18.84</td>
<td>42</td>
</tr>
</tbody>
</table>
The inhibition zone area was found to be dependent on the length of anode, as the clearing occurs all along the length of anode as shown in Table 4. This can be attributed to the fact that by increasing the anode length, the surface area of silver anode to which the bacterial species are exposed increases. Figure 14 represents the relationship between the anode surface area (by changing length) and the inhibition zone.

![Figure 14: Relationship between the inhibition zone area and the anodic surface area](image)

**Figure 14: Relationship between the inhibition zone area and the anodic surface area with anodic devices of 20 µA current, 6mm electrode separation, 6mm cathode length and 0.5 mm wire diameter**

**Effect of changing diameter:** To characterize the effect of varying the diameter, another test was performed by changing the anode diameter while keeping the length constant. Two different silver wires of diameter =0.5mm and 0.75mm were used. The length of anode was fixed at 10 mm and all other factors were kept same as in the previous case. The width of inhibition zone with 0.75 mm silver anode was more than that of 0.5mm silver anode, again suggesting that a larger surface area results in a larger inhibition area.
Statistical Analysis

To evaluate the effect of anode surface area on the inhibition area, a paired t-test was performed on the device with two different anodic areas. The t-test statistic was found to be 0.13 which rejects the null hypothesis and allows the conclusion that the area of anode is an important determinant for inhibition zone area. Thus it is concluded that the surface area of anode is an important factor governing the performance of the system.

The experiment results were then analyzed as a fully balanced factorial design. To analyze the model, an Analysis of Variation (ANOVA) was performed with three parameters- the current (log of current), surface area of anode (varying length of anode) and the separation between anode and cathode. The following table shows the ANOVA results for this design. The data used is given in Appendix in Table A.1, A.2 and A.3

Analysis of Variance (ANOVA) for inhibition zone area

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of anode</td>
<td>1</td>
<td>2645.0</td>
<td>2645.0</td>
<td>96.10</td>
<td>0.000</td>
</tr>
<tr>
<td>Log(current)</td>
<td>4</td>
<td>12212.0</td>
<td>3053.0</td>
<td>110.93</td>
<td>0.000</td>
</tr>
<tr>
<td>Separation</td>
<td>1</td>
<td>16.2</td>
<td>16.2</td>
<td>0.59</td>
<td>0.457</td>
</tr>
<tr>
<td>Error</td>
<td>13</td>
<td>357.8</td>
<td>27.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>15231.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Since the p-value for the separation is > 0.05, it is concluded that separation between anode and cathode is not a statistically significant predictor for the inhibition zone. This increase in separation from 6 mm to 15 mm did not affect the performance of the system much. The significant performance predictors were the surface area of the anode and the current. A regression analysis was performed with the area of anode and log(current) as the estimators of the inhibition zone.
Regression Analysis: Inhibition zone area versus surface area of anode and device current

To obtain the regression model, current data had to be normalized and scaled. Hence the current has been multiplied by a factor of 100 and the log of the resultant data is used for the regression analysis. The transformed data table (Table B.1) given in Appendix B was used to perform the regression analysis. The regression equation giving the relationship between the control variables, log (current) and surface area of anode, and the inhibition zone is given below:

Regression Analysis: Zone Area versus Log (Current*100), Surface Area

The regression equation is

\[ \text{Zone Area} = -51.3 + 11.9 \, \log (\text{Current*100}) + 2.30 \, \text{Surface Area} \quad \text{Eq}(5) \]

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>6221.2</td>
<td>3110.6</td>
<td>19.95</td>
<td>0.008</td>
</tr>
<tr>
<td>Residual Error</td>
<td>4</td>
<td>623.6</td>
<td>155.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>6844.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ S = 12.4865 \quad \text{R-Sq} = 90.9\% \]

Checking for any Interaction terms

This analysis was further extended to check if there was any kind of significant interaction between the parameters. The ANOVA result with an interaction term measuring interaction between current and surface area of the anode indicated that this interaction is not statistically significant (with a significance value of \( \alpha = 0.05 \)).

Regression Analysis: Zone Area versus Log (Current*100), Surface Area, Area*log(Current*100)

* Area*log(Current*100) is highly correlated with other X variables
* Area* log(Current*100) has been removed from the equation.

The regression equation is

\[ \text{Zone Area} = -51.3 + 11.9 \, \log (\text{Current*100}) + 2.30 \, \text{Surface Area} \]

The detailed regression analysis is given in Appendix B.
Thus, we conclude that the most important parameters that affect the performance of device significantly are the current rating of the device and the length of anode, which specifies the surface charge densities. The performance of the device is not affected with the separation between cathode and anode and the thickness of the anode.

The experimental results indicate that the proposed design for the prophylactic system works well and the parameters such as current and anode surface area affect the performance significantly. A selection criterion for various parameters is provided. For a particular system, improved performance values of these parameters can be obtained with the help of the regression model. It is recommended that for an anodic device with a 6-15 mm long anode with a wire diameter of 0.5mm, with the current value of 0.15 µA to 1.55 A and minimum separation of 5 mm can be used between anode and cathode providing the ions enough area to flow through the media. A device with a selection of parameters from these values is shown to give a good inhibition in Figure 15.

![Inhibition Zone](image)

**Figure 15:** Inhibition Zone with an anodic device having a 15mm X 0.5 mm anode, 6mm separation, 20 µA current and 6mm cathode.

The system demonstrated an effective prophylactic property. The performance of this prophylactic system was strongly dependent on the current and the surface area of the anode. This characterizes these two parameters as the critical design metrics. It has also been found that the performance of the device is independent of the separation between cathode and anode and the diameter of the anode.
Summary

This chapter presented the results obtained from the experiments conducted to determine the effect of variables affecting the performance of the proposed prophylactic silver ions system. The study identified the important control parameters which have a significant affect on the zone of inhibition, and the parameters which are not significant for the design of the system. Different statistical tests were performed to identify the significance of the control variables in the system. Finally, a regression model was presented to identify the parameter space of the variables which are critical for the performance of the system.
Chapter 6
Conclusions and Recommendations

This chapter provides a synopsis of this study discussing the impact of residual hardware device-based infections, and a need for a new antibacterial system. Design considerations for such a system and conclusions regarding the optimized parameters for effective working of the prophylactic system are also presented. Areas for future research which need to be examined for successful implementation of this system are also presented.

Conclusions

The increasing number of nosocomial infections due to residual hardware devices has become a challenging problem. The cost of mitigating the nosocomial infections associated with the residual hardware devices such as catheters, joint arthroplasties and fracture fixation devices in the United States has been increasing exponentially over time. Traditional treatment based on the use of antibiotics is losing ground because of the difficult and lengthy treatment processes involved, along with the increasing resistance exhibited by the pathogens. There is also an inherent problem with the nature of biomaterials used in these indwelling devices that provide some of the best adhesive surfaces for the bacteria to seed and grow. In light of all these factors, it is essential to identify an alternative system to kill the infection causing organisms in the most cost effective manner. Past studies have suggested the use of certain heavy metals as potentially antibacterial. Out of various materials studied, silver has been identified to be by far the most effective, safest, broadest antimicrobial metal when in its ionized form [Fuller 2005]. Silver has been proved to inhibit the growth of bacteria at relatively small local ion concentrations under the safe limits of use inside the human body.

In this study, an indwelling prophylactic system has been engineered using electrically ionized silver ions in a controlled environment and evaluated with respect to design parameters allowing for internal body constraints. These constraints include a
limit on the maximum local silver ion concentration (30 micrograms/milliliter) and a maximum lifetime exposure of 8.95 grams to the human body. The electrical current which can be used inside the body is limited to 20 micro amperes. The basic components of the system are a silver anode and cathode which are separated with insulation. The separation between the anode and cathode allows the silver ions to flow through the media containing microbes due to the voltage applied with a battery. The silver ions then act as a bactericidal agent within the local environment of the system and provide highly versatile antibacterial properties.

This study also identifies the critical design parameters, their performance and their improved performance values. It is concluded that the anodic end of the device is more conducive to bacterial inhibition. Amperage and the surface area of the anode are significantly important performance parameters of the prophylactic silver system. Performance is independent of the separation between cathode and anode. The analysis also shows that minimal currents (0.15 µA) can be used to get good bactericidal results. This analysis would be useful for the design of any indwelling device with prophylactic properties using silver ions.

The proposed system design can be incorporated for any implant either internal or external to the human body. The implant can be coated with silver metal in a micro-layer (thin-film) and the optimal parameters of anode area and amperage for effective device performance can be obtained from the mathematical model derived in this study.

**Recommendations for Future Work:**

- The bacteria may have the potential to become resistant to bactericidal action of silver ions. Further work would be required to investigate the possibility of the emergence of silver resistant strains of bacteria.

- The diffusion kinetics of silver ions from the anode to the cathode in the prophylactic system as well as preferential distribution of the inhibition zone around the
anode needs to be probed further. Investigation of the local concentration profile of silver ions across the length of the device can provide further insight into improving the efficiency of the system.

• Faraday’s law, operating under ideal conditions, has been utilized in this work to estimate the evolution of silver ions in the system. However, ideal conditions are not expected to exist in human tissue and hence the concentration of the liberated ions from the metal anode may deviate from the ideal calculations. This issue needs to be explored further.

• Finally the effect of silver on cellular structures such as osteoblasts and osteoclasts needs to be further investigated. Also, to validate this engineering model animal safety and efficacy studies will need to be performed before the device will be readily accepted for commercialization.
References:


Altman R. Colidal silver: where does it go when you drink it? How long does it stay in there? *Journal of the American Medical Association* (AMA) 1999


Boyd RF and Hoerl, BG. *Basic Medical Microbiology*. Published by Little, Brown, 1981


Canadian Institute for Health Information. 2002, Total knee and total hip replacements on the rise in Canada


Dibrov, P., J. Dzioba, K.K. Gosink, C. C. Hase; Chemiosmotic mechanism of antimicrobial activity of Ag+ in Vibrio Cholerae 2002 46:2668-2670
DiVincenzo GD, Giordano CJ, Schiever LS.; Biological monitoring of workers exposed to silver. *Internal Archives of Occupational Environment Health* 1985; 56;205-215


Donlan R.M., Biofilms: microbial life on surfaces; *Emerging Infectious Diseases* September 2002 Vol. 8, No. 9


Fuller, Thomas A. An analysis of the design of a prophylactic bactericidal implant: Concept to implementation. Masters Thesis 2005. Penn State University


Hill, W.R., and D. M. Pillsbury. Argyria, the pharmacology of silver. 1939. The Williams and Wilkins Co., Baltimore

Horner HC, Roebuck BD, Smith RP. Acute toxicity of some silver salts of sulfonamides in mice and the efficiency of penicillamine in silver poisoning. *Drug Chemistry and Toxicology* 1983; 6; 267-277

Jensen EJ, Rungby J, Hansen JC, Schmidt E, Pedersen B, Dahl R.; Serum concentrations and accumulation of silver in skin during three months treatment with an anti-smoking chewing gum containing silver acetate; *Human Toxicology* 1988; 7; 535-540


Miller K.E., Osteomyelitis Outcomes after Antimicrobial Therapy, *American Family Physician* 2004; 655-666


Rungby J. An experimental study of silver on the nervous system and on aspects of its general cellular toxicity. *Danish Medical Bulletin* 1990 37; 5; 442-449


Tweden K, Cameron JD, Razzouk A. Holmberg W, Kessy S. Biocompatibility of silver-modified polyesters for antimicrobial protection of prosthetic heart valves. *Journal of Heart Valve Disease* 1997; 6:553-561


Wataha JC, Lockwood PE, Schedle A, Noda M. Ag, Cu, Hg and Ni ions alter the metabolism of human monocytes during extended low-dose exposure. *Journal of Oral Rehabilitation*, 2002; 29; 133-139


APPENDIX A

Table A.1 Effect of varying device current on inhibition zone area

<table>
<thead>
<tr>
<th>External Resistor</th>
<th>Separation (mm)</th>
<th>Anode Surface Area (mm²)</th>
<th>Device Current</th>
<th>Inhibition Zone Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ω</td>
<td>6</td>
<td>31.4</td>
<td>1.5 A</td>
<td>110</td>
</tr>
<tr>
<td>75 KΩ</td>
<td>6</td>
<td>31.4</td>
<td>20 µA</td>
<td>70</td>
</tr>
<tr>
<td>100 KΩ</td>
<td>6</td>
<td>31.4</td>
<td>15 µA</td>
<td>60</td>
</tr>
<tr>
<td>1 MΩ</td>
<td>6</td>
<td>31.4</td>
<td>1.5 µA</td>
<td>40</td>
</tr>
<tr>
<td>10 MΩ</td>
<td>6</td>
<td>31.4</td>
<td>0.15 µA</td>
<td>20</td>
</tr>
</tbody>
</table>

Table A.2 Effect of varying electrode separation on inhibition zone area

<table>
<thead>
<tr>
<th>Separation (mm)</th>
<th>Anode Surface Area (mm²)</th>
<th>Resistance due to agar (KΩ)</th>
<th>External Resistor (KΩ)</th>
<th>Net circuit Resistance (KΩ)</th>
<th>Net current (µA)</th>
<th>Inhibition Zone Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>31.4</td>
<td>148</td>
<td>0</td>
<td>148</td>
<td>10.13</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>31.4</td>
<td>70</td>
<td>75</td>
<td>145</td>
<td>10.34</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>31.4</td>
<td>55</td>
<td>75</td>
<td>130</td>
<td>11.53</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>31.4</td>
<td>40</td>
<td>75</td>
<td>115</td>
<td>13.04</td>
<td>50</td>
</tr>
</tbody>
</table>

Table A.3 Effect of varying anode surface area on inhibition zone area

<table>
<thead>
<tr>
<th>Separation (mm)</th>
<th>Device Current</th>
<th>Anode Length (mm)</th>
<th>Anode Surface Area (mm²)</th>
<th>Inhibition Zone Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>20 µA</td>
<td>15</td>
<td>47.1</td>
<td>105</td>
</tr>
<tr>
<td>6</td>
<td>20 µA</td>
<td>10</td>
<td>31.4</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>20 µA</td>
<td>6</td>
<td>18.84</td>
<td>42</td>
</tr>
</tbody>
</table>
APPENDIX B

Table B.1 Transformed data to perform linear regression

<table>
<thead>
<tr>
<th>Current*100</th>
<th>Log (Current*100)</th>
<th>Anode Surface Area</th>
<th>Inhibition Zone Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1.176091259</td>
<td>31.4</td>
<td>20</td>
</tr>
<tr>
<td>150</td>
<td>2.176091259</td>
<td>31.4</td>
<td>40</td>
</tr>
<tr>
<td>1500</td>
<td>3.176091259</td>
<td>31.4</td>
<td>60</td>
</tr>
<tr>
<td>2000</td>
<td>3.301029996</td>
<td>31.4</td>
<td>70</td>
</tr>
<tr>
<td>150000000</td>
<td>8.176091259</td>
<td>31.4</td>
<td>110</td>
</tr>
<tr>
<td>2000</td>
<td>3.301029996</td>
<td>47.1</td>
<td>105</td>
</tr>
<tr>
<td>2000</td>
<td>3.301029996</td>
<td>18.84</td>
<td>42</td>
</tr>
</tbody>
</table>

Table B.2 Residuals and Fits values for the regression analysis

<table>
<thead>
<tr>
<th>Residuals</th>
<th>FITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15.0626</td>
<td>35.06255849</td>
</tr>
<tr>
<td>-6.92987</td>
<td>46.92986812</td>
</tr>
<tr>
<td>1.202822</td>
<td>58.79717775</td>
</tr>
<tr>
<td>9.720136</td>
<td>60.27986442</td>
</tr>
<tr>
<td>-8.13373</td>
<td>118.1337259</td>
</tr>
<tr>
<td>8.534753</td>
<td>96.46524681</td>
</tr>
<tr>
<td>10.66844</td>
<td>31.33155851</td>
</tr>
</tbody>
</table>
Figure B.1: Residual plots of zone area obtained from the regression analysis

Table B. 3 Residuals and Fits values of regression using interaction terms

<table>
<thead>
<tr>
<th>Log (Current*100)</th>
<th>Anode Surface Area</th>
<th>Area*Current</th>
<th>Inhibition Zone Area</th>
<th>RESIDUALS</th>
<th>FITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.176091259</td>
<td>31.4</td>
<td>36.92926553</td>
<td>20</td>
<td>-15.0625585</td>
<td>35.06256</td>
</tr>
<tr>
<td>2.176091259</td>
<td>31.4</td>
<td>68.32926553</td>
<td>40</td>
<td>-6.92986812</td>
<td>46.92987</td>
</tr>
<tr>
<td>3.176091259</td>
<td>31.4</td>
<td>99.72926553</td>
<td>60</td>
<td>1.202822252</td>
<td>58.79718</td>
</tr>
<tr>
<td>3.301029996</td>
<td>31.4</td>
<td>103.6523419</td>
<td>70</td>
<td>9.720135575</td>
<td>60.27986</td>
</tr>
<tr>
<td>8.176091259</td>
<td>31.4</td>
<td>256.7292655</td>
<td>110</td>
<td>-8.13372589</td>
<td>118.1337</td>
</tr>
<tr>
<td>3.301029996</td>
<td>47.1</td>
<td>155.4785128</td>
<td>105</td>
<td>8.534753188</td>
<td>96.46525</td>
</tr>
<tr>
<td>3.301029996</td>
<td>18.84</td>
<td>62.19140512</td>
<td>42</td>
<td>10.66844149</td>
<td>31.33156</td>
</tr>
</tbody>
</table>