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Developing an engineered antimicrobial/prophylactic system using electrically activated bactericidal metals

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Abstract The increased use of Residual Hardware Devices (RHDs) in medicine combined with antimicrobial resistant-bacteria make it critical to reduce the number of RHD associated osteomyelitic infections. This paper proposes a surface treatment based on ionic emission to create an antibiotic environment that can significantly reduce RHD associated infections. The Kirby-Bauer agar gel diffusion technique was adopted to examine the antimicrobial efficacy of eight metals and their ionic forms against seven microbes commonly associated with osteomyelitis. Silver ions (Ag^+) showed the most significant bactericidal efficacy. A second set of experiments, designed to identify the best configuration and operational parameters for Ag^+ based RHDs addressed current and ionic concentrations by identifying and optimizing parameters including amperage, cathode and anode length, separation between anode and cathode, and surface charge density. The system demonstrated an unparalleled efficacy. The concept was then implemented during in vitro testing of an antimicrobial hip implant, RHD.

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1 Introduction

Medical prosthetic devices, particularly joint replacement and fracture fixation devices are an indispensable component of modern medical treatment. Beginning in the early 1950s joint replacement surgeries increased as the surgical techniques and medical care associated with surgery improved. By the late 1980s, between 500,000 and 1,000,000 total hip replacements were performed per year [1]. In 2002, the Canadian Institute of Health reported that over the 6-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 while the total hip replacement surgery rate rose by 8.5% from 55.0 to 59.7 per 100,000 people [2]. In 2004, it was estimated that ~600,000 joint prosthesis and 2,000,000 fracture-fixation devices were inserted into patients in the United States. Ongoing advances in medicine have resulted in an increased number of RHD surgeries, but the total number of infections and the associated medical cost to mitigate the infections has not decreased. Infections associated with RHDs have significant economic and clinical significance. Every year in the United States there are approximately two million cases of nosocomial infections at a treatment cost approaching $11 billion. Of those infections, nearly half (45%) are associated with RHDs including catheters, joint arthroplasties, and fracture fixators [3–6]. For joint prostheses alone, an approximate incidence of 1–2% infections in 600,000 arthoplasties in 2004 resulted in an estimated infection mitigation cost of $360 million. This is the direct cost of mitigating the infection and it does not include lost time from employment or patient suffering. In 1991 the total cost of an infected patient, both in hospital and as an outpatient, was $45,000 as compared to the total cost of $8,600 associated with a non-infected patient [7].

Infection of a prosthetic hip costs approximately 5.23 times as much to treat when compared to a non-infected prosthesis. The approximate incidence of infection in the ~2,000,000 fracture fixation implantations in 2004 was 5%. The cost to mitigate these infections was ~$1.5 billion [4, 8]. Such high costs reflect the difficult and lengthy course of treatment for RHD associated infections.

The inherent risk of RHD associated infections results from a variety of interactions between the host and the microorganisms along with the concomitant factors related to implant surfaces, whether they are metallic, latex, silicone, or other forms [5, 9, 10]. The interactions explained further are with respect to the host body mechanism and microbial action. Briefly, disruption of epithelial and mucosal barriers and tissue trauma during device installation simultaneously triggers host immune responses and impairs host defense mechanisms [5, 11]. Once installed, conditioning films composed of host cells and cell products form a coating over the implant surface facilitating microorganism adhesion and colonization [10, 12–14]. Frequent sources of acute infection are opportunistic microorganisms present on epithelial surfaces. Surface seeding of RHDs may occur during installation, or as a result of microorganisms traversing incision sites or migrating along catheter surfaces, or via systemic spread following a septic condition [4, 5]. Although not all colonized RHDs become infected, those that do, involve biofilm formation [7]. Biofilms are complex sessile microcolonies of bacteria or yeast embedded within a microbially-derived protective extracellular polymeric matrix [4]. Once a biofilm forms, it is virtually inseparable from the implant [15]. Treatments for chronic infections involving biofilms are often rigorous as the biofilm matrix shields microorganisms from the action of macrophages and serves as a formidable antibiotic delivery barrier [5, 8]. Species frequently associated with RHD infections include: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Eschericia coli*, *Pseudomonas aeruginosa, Proteus mirabilis* and the fungus *Candida albicans* [16]. The problem is aggravated because many bacterial species are expressing resistance to antibiotics, thus rendering antibiotic treatment ineffective. Antibiotic resistance occurs more frequently in deep bone infections when the concentrations of antibiotic to the infected region are not sufficient to kill all of the bacteria. In many cases, the surviving bacterial species are able to adapt in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections [17–19].

Infections from RHDs are a multi-billion dollar/year medical issue in the U.S. alone. The pain suffering and deaths resulting from the infections are over and above the medical costs. Developing a system that can reduce or eliminate these infections could reduce medical costs by $1 billion a year—not to mention the personal suffering. Defining the characteristics and requirements for such a system is the focus of this paper. In the remaining sections, we will provide the engineering requirements for such a system.

2 Proposed concept

The approach introduced in this paper to reduce RHD associated infections is based on using controlled amounts of antimicrobial metals or metal alloys in their electrically generated ionic form. It is hypothesized that this would provide a safe and effective solution for eliminating or reducing infections associated with RHDs. Mader determined that in most cases, the antimicrobial efficacy of a metallic substance, particularly silver, is related to its ionic form [21]. Mader provided the understanding and rational behind the clinically limited use of metallic species as antimicrobial treatment [21, 22]. Historically, heavy metals have been used to treat microbial infections. In particular gold, silver and copper have been used in medicine as components of wound dressings, external skin treatments, and debridement agents for many years with anecdotal evidence of antimicrobial efficacy [23–26]. It is important to note that silver from metallic surfaces such as foils does not dissociate. Silver compounds that dissociate easily, such as silver nitrate, are locally sclerosing due to local silver ion concentrations exceeding local toxic limit to mammalian cells [27–29]. This has resulted in the limited use of metallics in a clinical setting (primarily for the prophylaxis of gonococcal ophthalmologic infections in newborns and in the treatment of burn patients and leg ulcers), and choosing an appropriate delivery system which can ionize silver and control the concentration of ions has been a significant problem.

Most metals in columns 11–14 of the Periodic Table can serve as antimicrobial agents. For our investigation, we eliminated bactericidals such as lead and mercury because they cannot be used inside the body in any form, and reduced the set of metals to silver, copper, titanium, gold, cadmium, nickel, zinc and stainless steel AISI 316L. Titanium and Stainless steel were included in the study because these are standard materials for use in indwelling applications. To test the efficacy of these metals, a method adopted from the Kirby Bauer agar gel diffusion technique was developed to determine the bactericidal/bacteriostatic efficacy of these eight metals and their electrically generated ionic forms. Each metal was tested against six 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: *Esherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis. Candida Albicans* was the only fungal species tested.

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 compared to most 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 an electrical potential is applied. This research supports the idea that ionization enhances the inherent antimicrobial activity of metals and increases their efficacy for treating infections. To quantify this effect, the number of ions generated by passing a given current across the metallic surface (governed by Faraday's law) and the corresponding effect on the growth of microbes was studied. Faraday's law states 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 properly controlled, this provides a unique mechanism for the controlled evolution of ions from the surfaces of noble metals. In an effort to ensure that the ionic form of the metal is delivered to the site of infection continuously, a circuit is configured to provide a controlled concentration of ions that is locally toxic to the bacterial species but at the same time, remains below total body/local tissue toxicity levels.

In the proposed design, under the effect of an electric potential, the ions pass over an insulated area via the media (agar, in this case) while moving from anode to cathode. Since the microbial species are contained in the media itself, they themselves become the metal ion carriers. Thus, the metal ions directly attack the microbial cells causing cell death. The performance of this system is dependent mainly on the control of the ionic concentration of metal ions and resistance of the system. The following factors are found to affect the ionic concentration and the surface charge density: current, anode–cathode separation and the surface area of exposed electrode.

3 Experimental methodology

The first part of this research was focused on identifying the metals and metal ions which can work as potential bactericides. A factorial nested experiment was designed to determine the materials and conditions that would provide the best inhibitory response for a variety of bacteria and fungi. Eight high purity metals and their electrically generated ionic forms were tested against six bacterial species and one fungus commonly associated with osteomyelitis. The microbial species and metals used in the experiment are listed in Tables 1 and 2 respectively. Independent variables in the experiment included microbial species, current, and particular metal used. The microbes were nested under metals and currents were nested under microbial species.

For this study, field isolates were used instead of ATCC cultures because of their easier availability and lower costs. Isolates of *Esherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis and Enterococcus faecalis* submitted to The Pennsylvania State University Animal Diagnostic Laboratory, and methicillin-resistant *Staphylococcus aureus* (MRSA) submitted to the J.C. Blair Hospital, Huntingdon, PA were diluted to a 0.5 McFarland standard and inoculated onto Mueller-Hinton agar plates (Remel, Lenexa, KS).

Metallic wires (Advent Research Materials Ltd.), of a uniform 1.0 mm diameter served as ion sources, specifically: silver (99.97% purity], copper (99.95+% purity), titanium (99.8% purity), gold (99.99% purity), cadmium (99.999% purity), nickel (99.98% purity), zinc (99.999% purity) and stainless steel AISI 316L.

Each metal wire was cut into pieces of 70 mm length. Approximately 31.6 mm of each 70 mm length was aseptically threaded through a single hole in a petri dish containing Mueller-Hinton agar. Once embedded in agar, 1 cm^2 surface area of each wire was exposed to the agar medium inoculated with the microbes. Each wire was connected to the positive lead of a 1.5 V AA battery holder with a battery, thus making it the anode. The cathode was created by connecting a bare wire of the same material as anode to the negative lead of the same battery. 2–3 mm at the distal end of this cathode wire was aseptically threaded into the Petri dish through a hole opposite the anode wire. The circuit was completed by adding a resistor in series with the anode. The complete in vitro testing configuration is shown in Fig. 1.

In order to calculate the ionic release, the following relationships were used. 31.6 mm of agar contact was measured, which provides for 1 cm^2 of contact surface area, based on calculations using Eq. 1.

$$\text{Surface area} = \pi r^2 + 2\pi rh = 1\,\text{cm}^2 \tag{1}$$

Equation 2 gives the Amperage calculation for a battery of 1.55 V incorporated with a 1.5 MΩ resistor. Using these parameters, approximately 1 μA current will be generated.

$$I = \frac{V}{R} = \frac{1.55\,\text{V}}{1.5 \times 10^6\,\Omega} = 1.03 \times 10^{-6}\,\text{A} \tag{2}$$

Control plates were run in each metal trial for all microbial species. The control circuit was designed as explained in

Table 1 Microbial species used during experimentation

Bacterial species	Gram (+)	*Staphylococcus aureus, Enterococcus faecalis, MRSA*
	Gram (−)	*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*
Fungus		*Candida albicans*

Table 2 Metals used during the experiments (metal and purity)

Metal	Purity
Silver	99.97%
Copper	99.95+%
Gold	99.99%
Titanium	99.8%
Nickel	99.98%
Zinc	99.999%
Cadmium	99.999%
Stainless steel AISI 316L	Alloy grade as determined by designation 316L

Fig. 1 but without any voltage or current source. All plates were incubated in air at 37°C for 24 h and examined for bacterial growth or zones of inhibition. For this experiment, five different current levels were generated to study the effect of current. The release of silver ions was in accordance with Faraday's law given in Eq. 3. For a silver wire carrying a constant current of 1 μA, 4.02 μg of silver ions will be liberated per hour (1 g equivalent of silver = 107.868 g Ag) as calculated from Eq. 3.

$$1\,\mu\text{A} * \frac{1\,\text{C}}{1\,\text{A}\cdot\text{S}} * \frac{1\,\text{F}}{96,487\,\text{C}} * \frac{107.868\,\text{g}}{1\,\text{F}} * \frac{10^6\,\mu\text{g}}{1\,\text{g}} * \frac{3600\,\text{g}}{\text{h}} = 4.02\,\mu\text{g/h} \tag{3}$$

The analyses of this study indicate that the metals, microbial species and current in the micro-amperes range

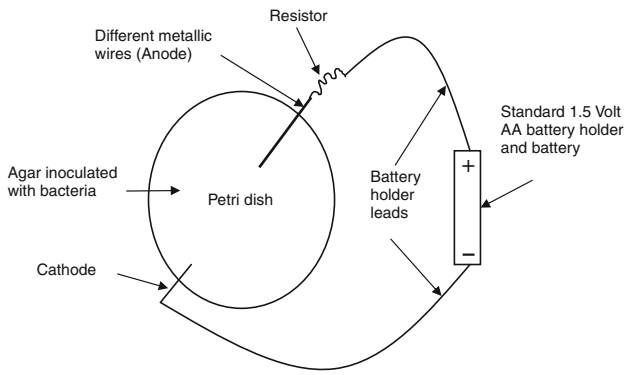

Fig. 1 Test configuration showing Petri dish filled with agar

were significant variables in determining the size of the inhibition zone. Literature review and preliminary tests also indicate that of all eight metals to be tested, copper ions, silver ions and cadmium were likely to show significant antimicrobial action. However, copper and cadmium cannot be effectively used for making RHDs since antimicrobial action of copper ions is limited to Gram positive species while cadmium has a toxicity profile associated with its in vivo use [30]. On the other hand, silver stimulated by electric current is expected to produce the large and consistent inhibition zones across a broad range of microbial species suitable for applications in RHDs,

Hence the second part of our study focuses on identifying factors that affect the prophylactic action of silver ions, designing and developing a system that can ensure proper delivery of silver ions to the site of infection, and evaluating the performance parameters of such a system. These factors are identified as the amperage of the device, and geometric parameters like the surface area of the anode (A_1), surface area of the cathode (A_3) and the separation between anode and cathode (L_2). The geometric parameters are shown in Fig. 2.

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

$$Z = F(A_1, L_2, A_3, \text{Amp})$$

where $A_1 = \pi DL_1$ and $A_3 = \pi DL_3$. The kill area is calculated by approximating the inhibition zone as a rectangle. The control and optimization of these factors was done by designing a fully balanced experiment (DOE) with both anodic and cathodic devices. The levels of different factors in the DOE were varied as follows:

- Current: 5 different currents were generated by using different resistors (between 75 kΩ and 10 MΩ) in series with a 1.5 V battery to study the effect of electric current. The resistance of the agar media itself was estimated to be approximately 250 KΩ.
- Anode–cathode separation: the insulation between the anode and the cathode was varied between 6.35 mm (¼″) to 25.4 mm (1″). This reflects the distance over which the media inoculated with the microbial species acts as a carrier of ions.
- Surface area of anode: This factor was varied by changing length (between 6 mm and 15 mm) and diameter of the anode wire (two diameters—0.5 and 0.75 mm).

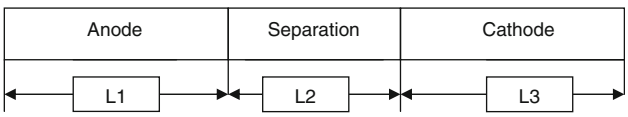

Fig. 2 Schematic describing the geometric parameters of the device

Table 3 Range of values of separation, current and anode length: the values shown below were used to perform various factorial experiments

Separation	Current	Anode length
	0.15 μA	
6.35 mm (¼″)	1.5 μA	6.0 mm
12.7 mm (½″)	15 μA	10 mm
25.4 mm (1″)	20 μA	15 mm
	1.5 A	

Results were used to perform an Analysis of Variance based upon each variable

The values of all the factors are given in Table 3. Note that the actual currents inside the system are expected to be lower than those given in Table 3, since each resistor is connected in series with the media, in the circuit.

The silver device used in these experiments is shown in Fig. 3. An insulated silver wire (99.97% purity) and silver paint (95% purity) were used to construct this device. The anode and cathode were integrated on the same wire and separated by a small insulation (See annotation in Fig. 3). The cathode was made by plating the insulated wire with silver paint. The insulation was stripped from the silver wire to expose the anode. Only *S. aureus* was used for inoculation in this experiment and the inoculation procedure was similar to that in the first part of the study. These devices at different factor levels were inserted in agar plates, incubated for 24 h at 37°C and studied for inhibition zone widths.

4 Results

Gold, titanium, zinc, nickel, and stainless steel did not inhibit growth of any bacterial species at any current level. However, cadmium and silver produced inhibition patterns across all electrically generated current levels. Additionally, cadmium required no current to create an inhibition pattern. The results of this study also support the hypothesis that silver ions are most affective against the growth of all the microbial species tested. A zone of bacterial growth inhibition was clearly visible for all bacterial species at all current levels greater than 0 μA when silver wire was used. Figure 4 shows the control and maximal inhibition zones for four different species and fungus. The widths of inhibition zones for the entire silver experiment are listed in Table 4. The average width of inhibition zone generated by silver ions across all species was 19.85 mm, measured perpendicular to the embedded wire. Zone widths for all tested species for all four currents were within one standard deviation (2.03 mm), of the mean. Copper also showed bactericidal properties, both in the ionic and metallic forms. Results for copper are shown in Table 5. For all Gram (+) species tested using copper, the inhibition zone width was directly proportional to the applied current. However, copper did not have any effect on Gram (−) or fungal species irrespective of the current. The average inhibition zone width for copper was 8.13 mm. Cadmium results are shown in Table 6.

A comparison of the inhibition zone widths, grouped by metals, across all currents used is shown in Fig. 5. It is evident that in the presence of small direct electrical current (few μA) for some of the heavy metals, all the bacteria and fungi species tested were unable to grow. The average inhibition zone width for silver across all factors and factor levels was 21.79 mm. An Analysis of Variance for the full nested design with five current levels nested within six different microbial species, nested below eight metals was performed using Minitab. The resulting ANOVA output is shown below:

Nested ANOVA: travel dist versus metal, microbe, current

Analysis of Variance for travel dist

Source	DF	SS	MS	F	*P*
Metal	7	12260.7333	1751.5333	24.800	0.000
Microbe	40	2825.0000	70.6250	5.450	0.000
Current	192	2488.0000	12.9583		
Total	239	17573.7333			

Effects of each factor in the DOE study on the bactericidal effect (inhibition zone width) are discussed below

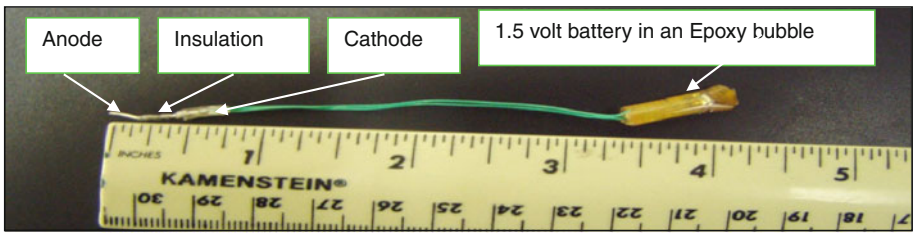

Fig. 3 Device design as used for the testing

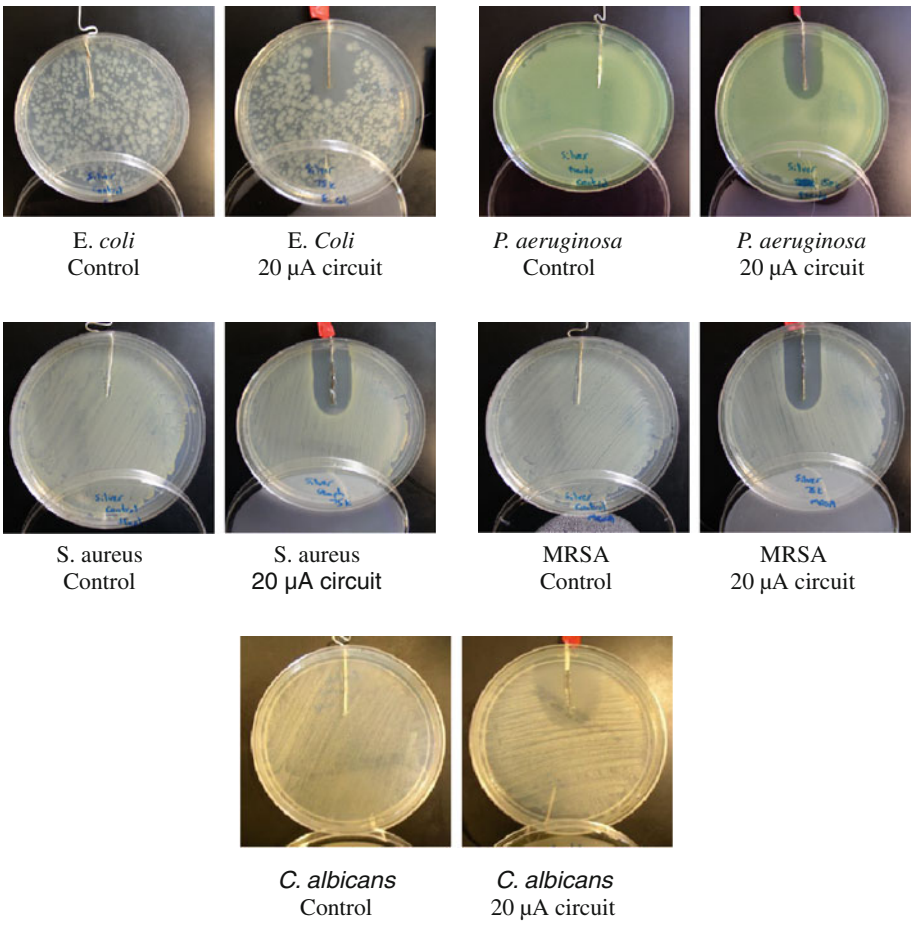

Fig. 4 Experimental results after preliminary testing using Silver

Picture results (control and 20 µA circuit)
Results for E. *faecalis were indiscernible due to the small size of photos when submitted for publication, however results are similar to those shown within this figure*

4.1 (a) Effect of circuit polarity

For this testing, the anode and cathode surface areas, electrode separation (5 mm) and current (1.5 A) were kept constant. Both the anodic and cathodic devices seemed to provide equally good inhibition against microbial growth. However, the inhibition zone was more clear and profound around the anode in both cases. No significant clearing was observed at the cathodes. It clearly proves that anodic end is more effective in producing a bactericidal environment. Figure 6 shows the cultured plates with inhibition zones for both anodic and cathodic devices in the agar media.

4.2 (b) Effect of electric current (amperage)

This in vitro testing was done using silver and copper anodic devices. Surface area of the electrodes, and the separation between them were kept constant. Length of the anode was 10 mm, and separation between the electrodes was 6 mm. The amperage factor was varied to study the effect of electric current on the system. The inhibition zone widths and areas are listed in Table 7 for silver and copper.

Figure 7 shows the cultured agar plates with current values as small as 15 µA. Width of the inhibition zones decreased noticeably when the current value changed from 1.5 A to 20 µA; the difference in the inhibition zone width between 20 µA and 15 µA was not significant. The zone width decreased noticeably at current values of 1.5 and 0.15 µA. Hence, the device amperage appears to be an important factor affecting the inhibition zone width. The device with 20 MΩ resistance (current = 0.075 µA) did not show an inhibition zone very clearly. Since the agar media itself has a resistance in the order of 250 KΩ, a current value of 0.15 µA obtained from using a 10 MΩ resistance is considered as the minimum required current for the device to work effectively against the concentration of 10^3 cfu/ml in the media. This also proves that the system works well even at a current value as low as 0.15 µA.

Table 4 Preliminary test results for silver using setup shown in Fig. 1

Current (μA)	Gram positive Ø in (mm)			Gram negative Ø in (mm)			Fungus Ø in (mm)
	S. aureus	*E. faecalis*	MRSA	*E. coli*	*P. aeruginosa*	*Proteus mirabilis*	*C. albicans*
0	6	0	0	5	0	0	0
0.5	18	17	18	20	18	7	34
1	20	19	18	21	21	8	30
10	20	21	18	25	21	10	32
20	20	20	18	24	22	12	30

All measurements are the diameters of bactericidal rings (in mm) measured outward from the central wire

Table 5 Preliminary test results for copper using setup shown in Fig. 1

Current (μA)	Gram positive Ø in (mm)			Gram negative Ø in (mm)		Fungus Ø in (mm)
	S. aureus	*E. faecalis*	MRSA	*E. coli*	*P. aeruginosa*	*C. albicans*
0	0	11	0	0	0	0
0.5	14	16	7	0	0	0
1	6	16	6	0	0	0
10	0	15	9	0	0	0
20	8	18	11	0	0	0

All measurements are the diameters of bactericidal rings (in mm) measured outward from the central wire

Table 6 Preliminary test results for cadmium using setup shown in Fig. 1

Current (μA)	Gram positive Ø in (mm)			Gram negative Ø in (mm)		Fungus Ø in (mm)
	S. aureus	*E. faecalis*	MRSA	*E. coli*	*P. aeruginosa*	*C. albicans*
0	8	5	14	6	(17)	28
0.5	6	6	13	5	(12)	28
1	8	6	13	4	(18)	31
10	6	5	15	6	(16)	30
20	7	5	16	5	(18)	30

All measurements are the diameters of bactericidal rings (in mm) measured outward from the central wire. Note (numbers contained within parentheses represent zones of decreased growth but lack complete clearing, indicating a reduction in growth but not complete inhibition)

The results from Table 7 indicate that the inhibition zone width is directly proportional to the applied current; lower the value of currents, the smaller the inhibition zone width. This can be attributed to the reduction in the concentration of silver/copper ions produced with decreasing amperage. It should be noted that this relationship is not linear, and more closely fits a log plot as shown in Fig. 8.

4.3 (c) Anode–cathode separation

The separation between the anode and cathode is the area where silver ions travel through the media, via the microbial species. This separation allows continuous pure silver ions supply from the device into the media. In studying the effect of electrode separation on the inhibition zone width, three separations were used—15, 10, and 6 mm. All the test devices had a 75 KΩ resistor in the circuit producing 20 μA of current. The length of anode was kept constant at 10 mm and the length of cathode at 6 mm.

Table 8 gives the results for the effect of electrode separation on inhibition zone width. These results indicate that varying separation between the electrodes does not have a significant impact on the inhibition zone. This can be explained on the basis of minimum current values necessary for silver ion generation and transport. Increasing the separation essentially causes an increase in resistance of the circuit. Since we have seen that the system works efficiently even at a current value as low as 0.15 μA, the change in resistance due to anode–cathode separation

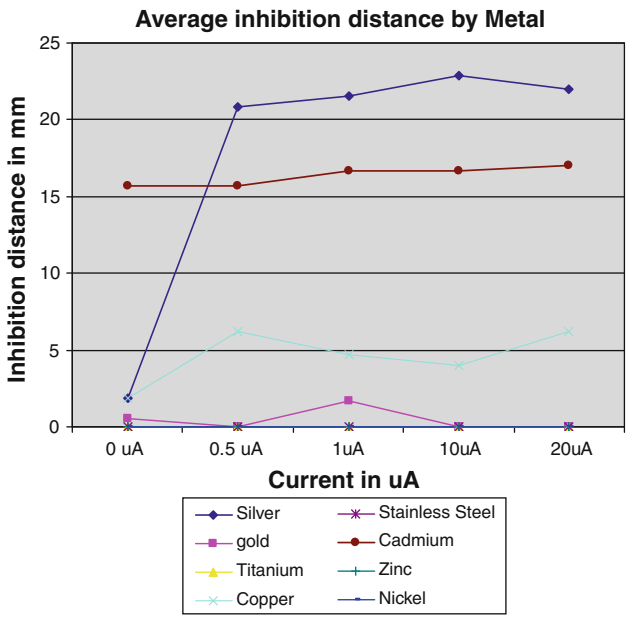

Fig. 5 Average inhibition zones for all eight metals calculated across all bacterial species and current levels

may not affect the system much. For the device design with which we are working, we suggest 5–10 mm to be a suitable separation distance between the electrodes.

4.4 (d) Surface area of anode

The two parameters that affect the surface area of anode (and hence the surface charge density) are length of the anode and diameter of the silver wire. These two were varied separately for this study. In the first test, a silver wire of diameter 0.5 mm was used with a cathode length of 5 mm and a separation of 5 mm between anode and cathode. A current of 20 μA was used. The anode length was varied between 6 and 15 mm. Table 9 shows the resulting inhibition zone areas. The inhibition zone area was found to be dependent on the length of anode, as the clearing occurs all along the anode length. This can be attributed to the fact that by increasing the length, we are increasing the surface area of silver anode to which the bacterial species are exposed. The larger surface area will result in a greater surface charge density, and hence a larger inhibition zone. There was no effect of changing the length (and hence the surface area) of the cathode from 6 to 15 mm on the inhibition zone width.

Another test was performed by varying the diameter of the anode wire but keeping its length constant. The length of anode was fixed at 10 mm and all other factors were kept same as in the previous case. Two different silver anode wire diameters were tested—0.5 and 0.75 mm. Width of the inhibition zone with 0.75 mm diameter anode

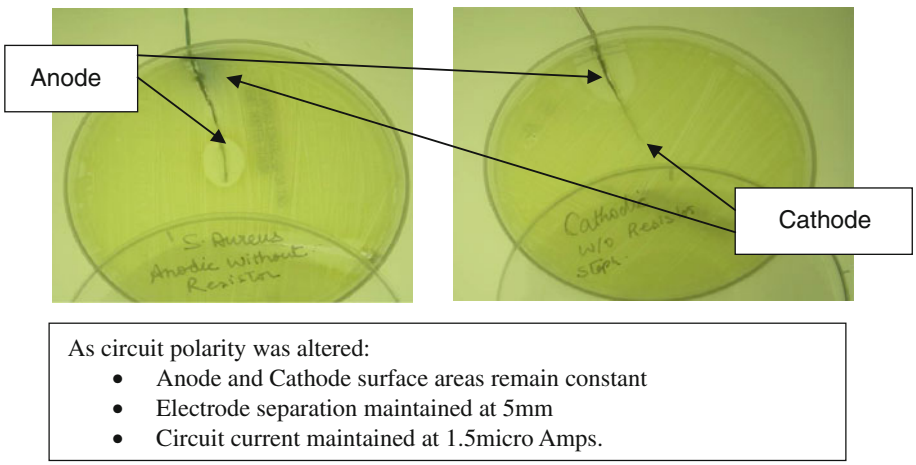

Fig. 6 Zone clearing for anodic and cathodic devices of 1.5 A. As circuit polarity was altered: anode and cathode surface areas remain constant; electrode separation maintained at 5 mm; circuit current maintained at 1.5 μA

Table 7 The zones of inhibition using different ionization currents for silver and copper

Current	Silver		Copper	
	Zone width (mm)	Zone area (mm^2)	Zone width (mm)	Zone area (mm^2)
1.5 A	11	110	8	80
20 μA	7	70	6	60
15 μA	6	60	5	50
1.5 μA	4	40	3	30
0.15 μA	2	20		

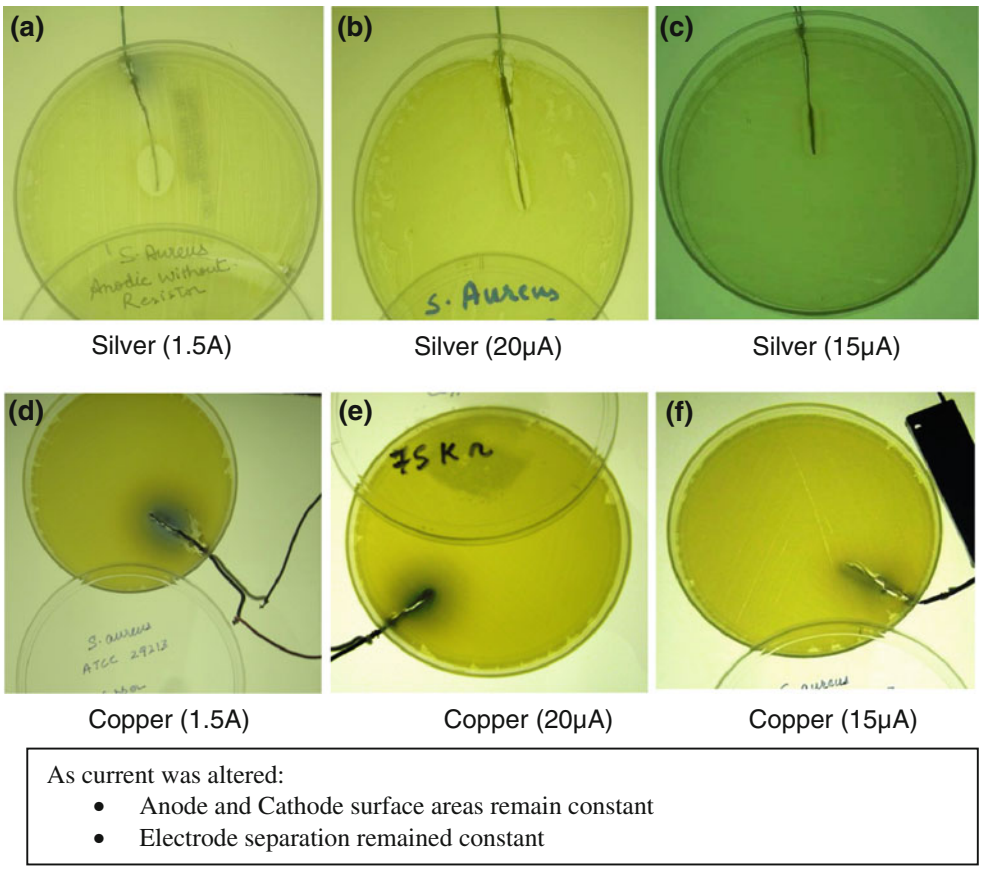

Fig. 7 Effect of current on microbial inhibition zone area with a silver device (**a**, **b**, **c**) and a copper device (**d**, **e**, **f**). As current was altered: anode and cathode surface areas remain constant; electrode separation remained constant

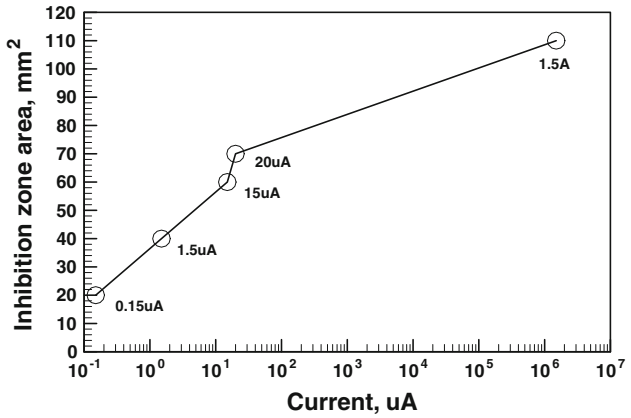

Fig. 8 Graph representing relationship between the inhibition zone area and the current with silver device

Table 8 Effect of anode–cathode separation on zone of inhibition

Separation (mm)	Zone width (mm)	Zone area (mm^2)
15	4	40
10	5	50
6	5	50

Table 9 Effect of anode surface area on zone of inhibition

Anode length (mm)	Surface area (mm^2)	Zone area (mm^2)
15	47.1	105
10	31.4	70
6	18.84	42

was more than that with the 0.5 mm diameter anode. From the results of the statistical analysis, the area of anode appears to be an important determinant ($P = 0.13$) for the area of inhibition zone. To characterize this effect, this experiment was repeated by keeping the surface area constant. The area of the inhibition zone was found to be equal in both the cases. Thus it can be concluded that surface area of the anode is an important factor in predicting performance of the system.

4.5 (e) Statistical inference

The experimental results were then analyzed as a fully balanced factorial design. An ANOVA was performed with

three parameters—the current, length of anode [or the surface charge density] and the separation between anode and cathode. The ANOVA ($\alpha = 0.05$) results obtained for this design, in Minitab, are presented below.

Analysis of Variance for zone area

Source	DF	SS	MS	F	*P*
Area of anode	1	2645.0	2645.0	96.10	0.000
Current	4	12212.0	3053.0	110.93	0.000
Separation	1	16.2	16.2	0.59	0.457
Error	13	357.8	27.5		
Total	19	15231.0			

Since the *P*-value for separation is large, it is concluded that separation between anode and cathode is not a statistically significant predictor of the inhibition zone. The increase in separation does not affect the performance of the system much. The significant performance predictors are the surface area of the anode and the current. This is in accordance with the experimental observations discussed earlier. Further, a regression analysis was performed with the area of anode and current as estimators of the inhibition zone. The Minitab output is shown below.

Regression analysis: zone versus surface area of anode and current

The regression equation is zone = 134 − 23.0 area of anode − 17.3 current

Analysis of Variance

Source	DF	SS	MS	F	*P*
Regression	2	14685.9	7342.9	229.00	0.000
Residual error	17	545.1	32.1		
Total	19	15231.0			

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

Experimental observations and statistical results indicate that the proposed design for the prophylactic system is highly effective. The performance of this system is highly dependent on the current and surface area of the anode, which specifies the surface charge density. However, performance of the system appears to be independent of the separation between cathode and anode and diameter of the anode.

For a particular system, optimized values of the significant design parameters can be obtained from the regression model. It is recommended that for a device with a 6–15 mm long anode of 0.5 mm diameter, a current value of 0.15 μA to 1.55 A, a minimum separation of 5 mm be used between anode and cathode to provide the ions sufficient area to flow through the media. A device with these design parameters is shown to give a significant inhibition zone as seen in Fig. 9.

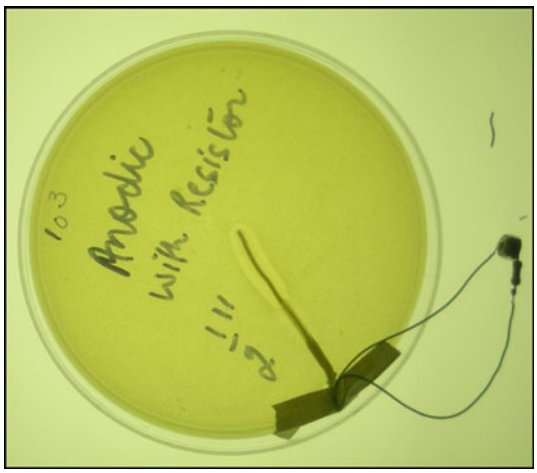

Fig. 9 Inhibition zone with anodic device having a 15 mm × 0.5 mm anode, 6 mm separation, 20 μA current and 6 mm cathode

5 Conclusions and future work

This study has identified the materials, conditions, and operational specifics associated with inhibition of a broad set of bacteria and fungi, along with a suitable design, which can be incorporated in developing a prophylactic RHD. A broad set of bactericidal metals were tested to determine inhibitory effects on various types of bacteria and fungi. Silver was by far, the most effective, safest, broadest antimicrobial metal when in its ionized form. An engineering system harnessing the potential of silver ions in a controlled evolution, allowing for internal body constraints was also successfully engineered.

This study is the first in a series that utilizes an electrically controlled power source in order to control the generation of ions from metallic surface with the overall constraints of use within a RHD. This paper proposes design of such a system that allows for any implant, either internal or external to the human body, to be coated with silver metal in a micro-layer (thin-film). The system generates silver ions continuously and effectively, with anode and cathode separated with an insulation which allows the ions to flow through the media containing microbes. The silver ions then act as a bactericidal agent within the local environment of the implant and provide a highly versatile antibacterial property. As the results indicate, not only is

Fig. 10 A bactericidal hip replacement in agar in *Pseudomonas aeruginosa*

the engineering control of the implant possible today but the ionic effects within culture are unparalleled in antimicrobial culture work.

The study also identifies the critical design parameters and their performance values for such an antimicrobial system. It is concluded that amperage value and the surface area of the anode are significantly important performance parameters of the prophylactic silver system, and the system performance is independent of the separation between cathode and anode and the thickness of the anode. The analysis also shows that minimal current (0.15 μA) can also be used to get good bactericidal results with this particular device. This analysis would be useful for design of the proposed prophylactic system using silver ions. Using the results obtained from the experiments described in this paper, a hip replacement implant as shown in Fig. 10 was designed. Figure 10 also shows an in vitro test result with the inhibition zone around the implant when placed in agar media containing *Pseudomonas aeruginosa.*

Further studies are needed to examine the direct cellular effects of silver ions on the cell lines like osteoblasts and osteoclasts. If this engineering model holds true, animal safety and efficacy studies will need to be performed before the model will be readily accepted for commercialization.

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