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Biocompatibility analysis of an electrically-activated silver-based antibacterial surface system for medical device applications

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Abstract The costs associated with the treatment of medical device and surgical site infections are a major cause of concern in the global healthcare system. To prevent transmission of such infections, a prophylactic surface system that provides protracted release of antibacterial silver ions using low intensity direct electric current (LIDC; 28 μA system current at 6 V) activation has been recently developed. To ensure the safety for future in vivo studies and potential clinical applications, this study assessed the biocompatibility of the LIDC-activated interdigitated silver electrodes-based surface system; in vitro toxicity to human epidermal keratinocytes, human dermal fibroblasts, and normal human osteoblasts, and antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli* was evaluated. The study concluded that the technological applications of the surface system for medical devices and surgical tools, which contact human tissues for less than 1.5 h, are expected to be self-sterilizing without causing toxicity in vivo.

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1 Introduction

As reported to the Centers for Disease Control and Prevention (CDC) between 2006 and 2008, the most performed surgical procedures were, in decreasing order of magnitude, orthopedic, cardiac, obstetric and colorectal [1]. In fact, over the past ten years the number of yearly joint replacement surgeries in the United States has more than doubled, from 451,000 in 2000 to 1 million in 2009 [2, 3]. Furthermore, it is estimated that there are approximately 1.7 million people in the US living with limb loss, which makes them ideal candidates for prosthetics or orthotics [4]. Unfortunately, the risk of surgical site infection (SSI) associated with surgical procedures is still significant. For the procedures reported to the CDC between 2006 and 2008, there were 16,147 SSIs following 849,659 operative procedures [1]. The averaged incidence was as high as 5.6 % for colorectal surgeries, but the study concluded an overall SSI rate of 1.9 % [1]. In the case of joint replacement surgeries alone, SSI occurs in 0.8–1.9 % of knee arthroplasties and 0.3–1.7 % of hip arthroplasties [5]. In addition to the pain, suffering and deaths associated with SSI, the costs of mitigating treatments for SSI are enormous. Specifically, the average cost of care for prosthesis removal, 6 weeks of parenteral antibiotics, and prosthesis reimplantation is 300 % higher than that of

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non-infected patients, and in some cases these costs may be higher than the expense of the original joint replacement surgery [6]. These high costs are caused by prolonged length of stay, hospital readmission, additional infection therapies and surgeries, not to mention the difficulties in treatment of infection caused by antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*. Indeed, the most common bacterial strain responsible for surgical infection is *S. aureus*, and the only method employed to avoid infection is through strict adherence to sterile surgical conditions and through the administration of prophylactic antibiotics [7].

An emerging alternative solution to prevent the occurrences of SSI is to design the surfaces of implants and medical devices to be inherently antibacterial. For example, in a trial involving patients with infection associated with hip arthroplasty, the use of a vancomycin-loaded spacer (as compared with no spacer) resulted in a decreased rate of recurrent infection (11 vs. 33 %, $P = 0.002$) [8]. In another study, an antibiotic-loaded mineralized collagen coating was evaluated for use with titanium implants; it was shown that the coatings were stable with good cytocompatibility and a reasonably good antibacterial effect [9]. However, the overuse of modern antibiotics has revealed the emergence of antibiotic-resistant microorganisms, and prescribes the need for improved antimicrobials that exhibit a lower incidence of resistance. Appropriately, the antibacterial activity of silver and silver products has been widely investigated and implemented into medical devices in various forms for thousands of years with very little resistance [10]. In addition to having broad antimicrobial activity against Gram-negative and Gram-positive bacteria, fungi and antibiotic-resistant organisms, the ability of bacteria to develop resistance to silver, silver nanoparticles, or silver ions is low due to its multimodal antibacterial activity [11–13].

However, the clinical use of silver for in vivo applications has been limited due to the lack of an appropriate delivery system for the controlled release of silver ions (Ag^+). Past studies have focused on metallic silver coatings for catheters and orthopedic fixation pins [14–16]. Although bacterial colonization was significantly reduced for the silver-coated catheters in vitro [14], in vivo studies with silver-coated fixation pins failed to demonstrate decreased bacterial adhesion [15, 16]. It is likely that these silver coatings lacked antibacterial efficacy due to their inability to actively release Ag^+. Alternatively, a wound dressing that utilized silver nanoparticles was capable of releasing sufficient silver to inhibit bacterial growth, yet the biocompatibility of the system was not evaluated [17]. To address these issues, a surface system utilizing low intensity direct electric current (LIDC) was employed in this study as an effective mechanism for the continuous release of Ag^+ into a surgical infection site. Two different configurations of this system have demonstrated basic antibacterial efficacy against both Gram-positive and Gram-negative bacterial strains in vitro [18–20]. However, prior to the extension of this technology into multiple different in vivo surfaces of medical devices and implants, the safety and efficacy of the system requires in vitro cytotoxicity evaluation to mammalian cell lines. Therefore, the objective of this study was to assess the biocompatibility of the prophylactic surface system that uses electrical activation as a delivery mechanism for antibacterial Ag^+. Specifically, investigations of the cytotoxicity to human epidermal keratinocytes (HEK), human dermal fibroblasts (HDF) and human osteoblasts (OST), and the antibacterial efficacy to *Escherichia coli* and *S. aureus* of electrically liberated Ag^+ from interdigitated silver ink electrodes at specific current and voltage levels within a range of electrical parameters previously determined to kill the bacterial strains were conducted [18–20].

2 Materials and methods

2.1 Surface system design

The antimicrobial surface system consists of an interdigitated pattern of alternate electrically charged positive and negative silver-based electrodes separated by an electrically resistive insulation, and activated by LIDC. The system functions on the principle of oligodynamic iontophoresis; when the surface is in contact with any conducting liquid, the LIDC in the system causes the release and diffusion of antibacterial Ag^+ from the silver anodes into the liquid medium. Surfaces in healthcare environments (e.g. medical implants, surgical tools) are frequently exposed to bacteria in the form of aqueous liquid media such as blood or other body fluids. In presence of such infected media on the surface, the resulting electrically stimulated Ag^+ trigger the system to self-sterilize. This surface system design and its working principle are explained in more details elsewhere [19, 20].

In this study, the primary experimental apparatus consisted of the interdigitated silver-based surfaces and their electrical circuits. Electrodes were printed with #125-15 silver ink (Creative materials; Ayer, MA) onto the bottom membranes of BD Falcon™ Transwell inserts for six-well plates with 0.4 μm pores (BD Biosciences; Bedford, MA) using a 4th generation Envisiontec™ 3D-Bioplotter (EnvisionTEC GmbH, Germany). The pattern dimensions and printing parameters are presented in Table 1. Following printing, the inserts were baked at 60 °C for 1 h to cure the ink and allow degassing of binders in the ink. They were then connected to an Enercell® 6 V battery (Radioshack;

Table 1 Antimicrobial surface system design parameters

Parameter	Value
Pattern diameter	25 mm
Electrode width	0.72 mm
Electrode separation	0.48 mm
Total number of electrodes	19
Electrode thickness (height)	15 μm
Nozzle diameter	0.26 mm
Nozzle velocity	4 mm/s
Printing pressure	4 bar

Fort Worth, TX) via an electrical resistor circuit that resulted in a system current of 28 μA. Finally, the Transwell inserts with the electrically activated interdigitated electrode pattern on their membranes were suspended into the cell media in six-well culture plates. In this manner, the conventional in vitro cell culture model was preserved, and any cellular inhibition could then be attributed to the silver ink, the electrical current or Ag^+, and not due to the lack of a surface for cell attachment. A schematic of the electrode testing configuration and an actual Transwell insert are shown in Fig. 1a, b.

Prior to cell culture testing, the inserts were rinsed with deionized (DI) water, and sterilized by UVB in the cell culture hood. The printed electrode surfaces were subjected to a minimum of 1 h light exposure and rotated 90°, for a minimum of 4 h total exposure and complete 360° coverage. The electrodes were then rinsed in sterile Gibco® Hank's balanced salt solution (HBSS; Life Technologies; Grand Island, NY) and in cell culture medium, and then equilibrated in cell culture medium in the incubator during cell harvesting.

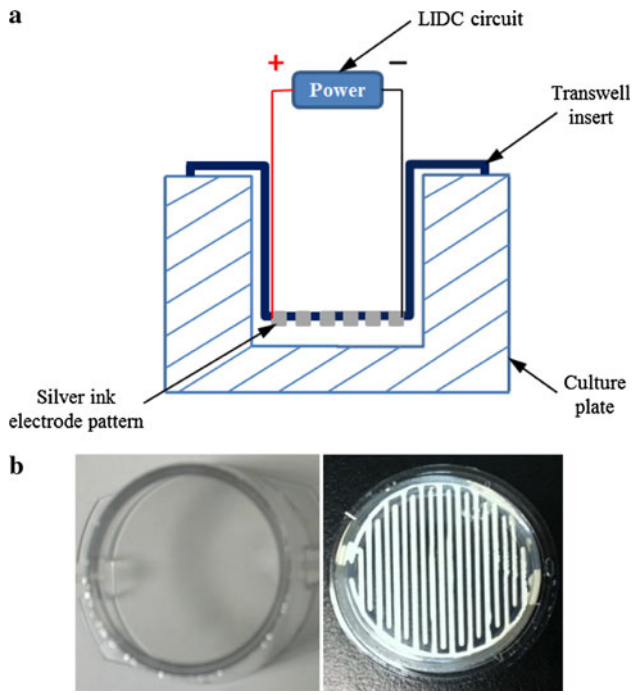

Fig. 1 **a** Schematic of electrode testing configuration in a cell culture plate. **b** Blank Transwell insert (*left*) and silver ink printed onto the bottom of Transwell insert (*right*)

2.2 Biocompatibility testing

Neonatal human epidermal keratinocytes (HEK), neonatal human dermal fibroblasts (HDF), and normal human osteoblasts (OST) (Lonza; Walkersville, MD) were grown in keratinocyte growth medium-2, fibroblast growth medium and osteoblast growth medium (Lonza, Walkersville, MD) respectively, in 75 cm^2 cell culture flasks to approximately 80 % confluency in a 37 °C humidified 5 % CO_2 incubator. Cells were then passed into six-well cell culture plates (150,000 cells/well) for cytotoxicity and antimicrobial testing. Each cell culture well either had an electrode printed onto the bottom of a Transwell insert to fit into the well (with and without 28 μA current) or remained devoid of electrodes and served as a well control.

Following seeding, cells were grown overnight until they reached approximately 70–80 % confluency. Transwell inserts were inserted into the wells (with or without 28 μA current), and incubated for a fixed time interval. To mimic short surgical procedure durations, an incubation interval of 1.5 h was employed in the study. The general layout for these experiments may be observed in Fig. 2.

To assess cell viability, alamarBlue® (aB) (Life Technologies; Grand Island, NY) viability assay was used. At the conclusion of the experimental time point, the current

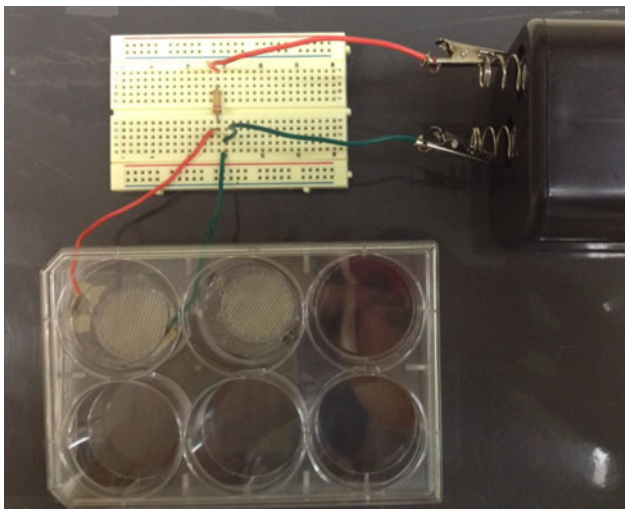

Fig. 2 Overview of apparatus, showing six-well plate, battery and waffle board, all mounted on an acrylic plate. Silver electrodes printed onto the bottom of Transwells in *upper left* well (with 28 μA current applied) and in *upper middle* well (no current applied), with an open well control in *upper right*

electric circuit was turned off, the media was replaced with aB solution (10 % aB dye in cell culture medium), and the HEK, HDF, and OST were incubated for 3 h. The solution was transferred from the six-well plates to a black 96-well plate (100 µl per 96-well) and the fluorescence quantitated at 560EX nm/590EM nm.

2.3 Antibacterial efficacy testing

Tryptic soy (TS) broth and agar (Difco Laboratories, Detroit, MI) was used as the bacterial cultivating medium for *E. coli* MG1655 and *S. aureus* ATCC 25213. Isolated bacterial colonies were grown overnight at 37 °C from frozen samples on an agar plate. An isolated bacterial colony was vortexed and diluted to 10^3 CFU/ml in TS broth, and seeded into six-well plates as described previously for mammalian cell culture (Fig. 2). To ensure quality control and for seeding accuracy, bacteria were diluted in phosphate buffered saline (PBS; calcium/magnesium free, Life Technologies, Grand Island, NY) at 10^3, 10^2, 10^1, and 10^0 and plated overnight.

To assess the antibacterial efficacy of the silver electrodes, each bacterial strain was exposed to the "off" electrodes for 1.5 h, and the "on" electrodes for 0.5 h, 1.0 h, or 1.5 h at which point all electrodes were removed from the treatment wells. Bacterial cell counts were assessed on titer plates at 0.5, 1.0, 1.5, 2.5, 3.5, and 5.5 h after the onset of the experiment.

2.4 Statistics

The mean viability data was calculated by normalizing each of the electrodes to the well control. Significant differences ($P < 0.05$) were determined using the PROC GLM procedure (SAS 9.2 for Windows). When significant differences were found, comparisons were performed using Dunnett's t test at $P < 0.05$ level of significance. All experiments were performed in triplicate ($n = 3$ per treatment group) with two samples of each type in each experimental run.

3 Results

Incubation of cells, with or without 28 µA current, with printed Transwell inserts for 1.5 h showed no significant decrease in viability compared to control for all cell types, except for HEK exposed to the "off" electrodes (Fig. 3).

For the antibacterial efficacy experiment, both *E. coli* and *S. aureus* strains were affected by the silver electrodes with and without current applied (Figs. 4, 5).

For *E. coli* at the conclusion of the experiment, 0.5 h "on" exposure inhibited bacterial growth similar to 1.5 h "off" exposure, 1.0 h and 1.5 h "on" exposure both resulted in inhibition past the limit of detection (data points of "0" at 5.5 h not displayed), and control cells displayed the characteristic log phase growth (Fig. 4). For *S. aureus,* 0.5 h "on" exposure inhibited bacterial growth similar to 1.5 h "off" exposure, 1.0 h "on" exposure further increased inhibition, 1.5 h "on" exposure resulted in inhibition past the limit of detection (data points of "0" at 5.5 h not displayed), and control cells displayed the characteristic log phase growth (Fig. 5).

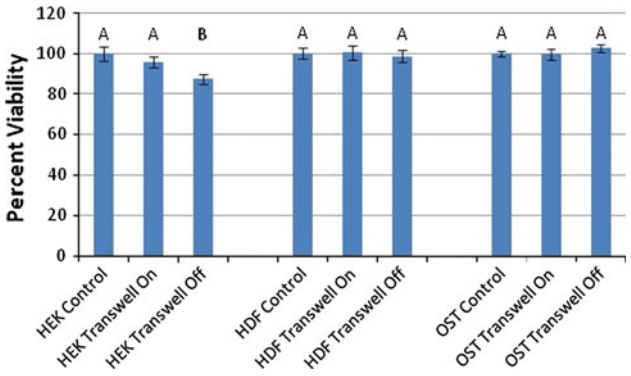

Fig. 3 Viability of HEK, HDF, and OST following 1.5 h exposure to Transwell electrodes, with and without 28 µA current; viability normalized to respective cell control. *Different letters* denote statistical difference ($P < 0.05$) in viability between treatment groups

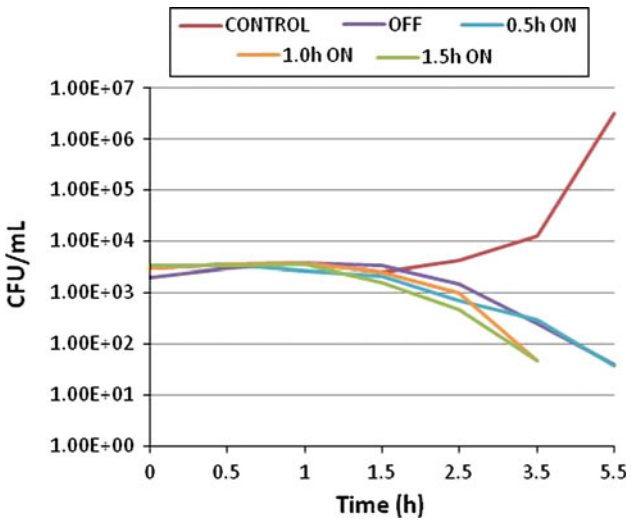

Fig. 4 Bacterial killing curves for *Escherichia coli* exposed to silver electrodes, with or without 28 µA current, for up to 1.5 h

4 Discussion

Despite advances in SSI control practices, including improved operating room ventilation, sterilization methods, surgical technique, and administration of prophylactic

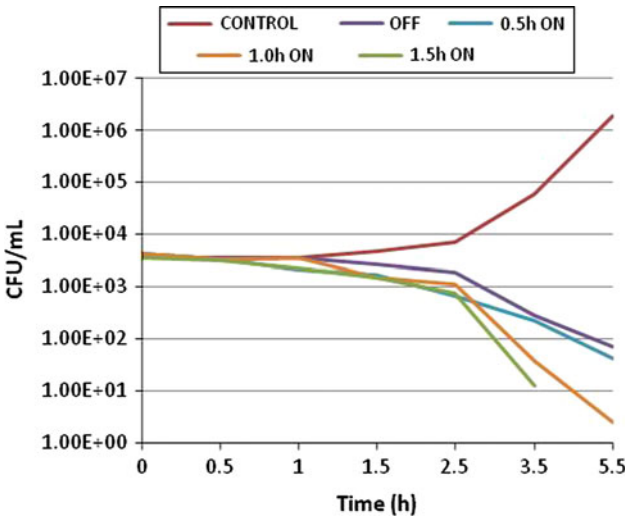

Fig. 5 Bacterial killing curves for *Staphylococcus aureus* exposed to silver electrodes, with or without 28 μA current, for up to 1.5 h

antimicrobials, SSIs remain a significant cause of morbidity and mortality among hospitalized patients. These SSI can occur following any operative procedure, and may occur within 30 days of surgery or within one year of surgical implant. Because most US hospitals are now reimbursed at a flat rate for surgical procedures, a SSI is likely to result in uncompensated costs for additional surgical procedures, treatments and readmissions [6]. The potential cost savings if SSIs can be prevented are significant, in addition to the improved patient outcome and consequent decreased mortality rate.

Silver-based technology has the greatest potential for impact in SSI reduction because of its broad-spectrum antimicrobial activity and lower incidence of bacterial resistance than conventional antibiotics [11–13]. Medical applications that take advantage of the antibacterial activity of silver have been complicated by a lack of release of silver from their systems or have not evaluated their biocompatibility with the target tissue [14–17]. Additionally, there is general agreement that Ag^+ are responsible for the biological action that is especially pronounced against microorganisms. Therefore, the ability of electrically activated silver electrodes to slowly release these ions from their surface over time makes them especially attractive for surfaces on both high-contact patient areas as well as medical and surgical tools. However, the lethal concentration of Ag^+ to human cell lines such as germ-line stem cells, dermal fibroblasts, and keratinocytes is generally below 5 μg/ml, [21–24].

In this study, interdigitated silver-based electrodes were utilized in an LIDC system in order to provide protracted antimicrobial Ag^+ release, as previously demonstrated [19, 20]. However, before the electrode may be used in vivo, it is essential to assess the toxicity to the targeted tissues in vitro. For preliminary biocompatibility experiments, electrodes were printed directly onto the bottom of six-well culture plates. However, it was noted that their location inhibited the attachment and proliferation of cells, and also forced the onset of exposure to immediately following cell seeding. Because each of the cell types tested in this study are adherent cells, the location of the printed electrodes was moved to the bottoms of Transwell inserts, so that the cells were allowed to attach, proliferate and reach 70–80 % confluency in the cell culture plates prior to the initiation of electrode exposure (Figs. 1, 2). The study showed that 1.5 h exposure to the electrodes did not induce significant cytotoxicity to HEK, HDF, or OST except for "off" electrode exposure to HEK (Fig. 3). This data suggests that acrylates from the silver ink or passive Ag^+ release is minimal following 1.5 h exposure of cells to the Transwell printed electrodes, and that the HEK were more sensitive to this occurrence than HDF and OST.

The antimicrobial efficacy of the electrodes was also evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli.* It was shown that the 1.5 h exposure to the electrodes was necessary to inhibit *S. aureus* while only 1.0 h was necessary to inhibit *E. coli* past the limit of detection (Figs. 4, 5). The thicker cell wall of *S. aureus* than *E. coli* is likely the reason for the required increased exposure time. The antibacterial efficacy of the electrodes was also evaluated against a Ag-resistant *E. coli* (J53(pMG101)), and it was noted that 1.5 h exposure did not significantly inhibit its growth compared to control for either "on" or "off" electrodes (data not shown). This bacterial strain served as not only a silver control, but also a 28 μA current control, and shows that neither the "off" electrode nor the current itself affected the bacteria significantly compared to control.

5 Conclusions

To prevent transmission of infections from medical devices and surgical sites, an LIDC-activated prophylactic surface system that provides protracted release of antibacterial silver ions was recently developed. To ensure the safety for future in vivo studies and worth for potential clinical applications, the biocompatibility of the interdigitated silver electrodes-based surface system was assessed. The system showed good antibacterial efficacy against both Gram-positive and Gram-negative bacterial strains by completely inhibiting the growth of *S. aureus* and *E. coli* after 1.5 h of exposure. Furthermore, the system displayed good biocompatibility over this time period with human epidermal, dermal, and skeletal cells, representatives for application target tissues, by only slightly decreasing the viability of HEK. The applications of this promising

technology are particularly relevant for high contact surface areas that are prone to microbial contamination such as stethoscopes, hospital bed rails, door handles and elevator buttons, and also for surgical tool surfaces such as scalpel handles, endoscopes, and forceps.

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Conflict of interest The authors declare that they have no conflict of interest.

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