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Regular Article

# Particle assisted removal of microbes from surfaces

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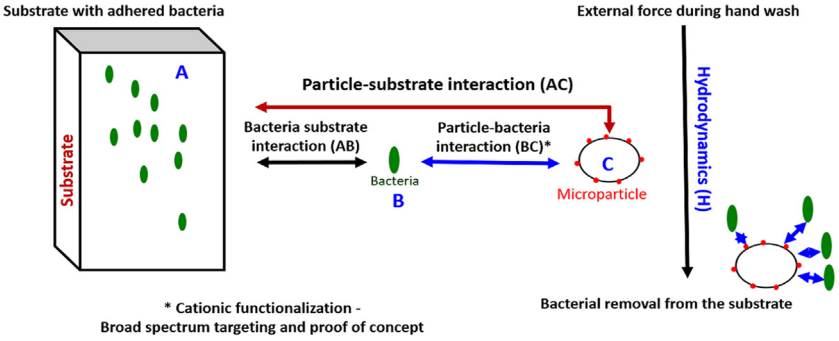
## GRAPHICAL ABSTRACT

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## ABSTRACT

Increased reliance on kill based approaches for disinfection raises concerns of antimicrobial resistance development and has significantly elevated the need for alternate approaches for skin and substrate disinfection. This study focuses on reducing harmful microbes from substrates primarily via removal and to a lesser extent by kill.

*Hypothesis:* Functional micro-particles designed to adhere to microbes, with a force greater than the force of microbial adhesion to the substrate, would result in enhanced removal-based disinfection of substrates when subject to an external force.

*Experiments:* Silica particles were functionalized with a cationic polymer to bind strongly with bacteria via Coulombic interactions. Disinfection efficacies of substrates with functional particles and control groups were evaluated under conditions relevant for handwashing.

*Findings:* Functionalized silica micro-particles result in ~4 log reduction of *E. coli* from an artificial skin substrate in 30 s as compared to a maximum of 1.5 log reduction with control particles. Bacterial viability assays indicate a mechanism of action driven by enhanced removal of bacteria with minimal kill. Particle number density, size and suspension velocity along with strong particle – bacteria interactions have been found to be the primary factors responsible for the enhanced bacterial removal from surfaces.



*Abbreviations:* SP0.2, Silica-PEI 200 nm; SP 0.6, Silica-PEI 600 nm; SP2, Silica-PEI 2 μm; SP10, Silica-PEI 10 μm; SP50, Silica-PEI 50 μm; SP200, Silica-PEI 200 μm; SC10, Silica-control 10 μm; SC50, Silica-control 50 μm.

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## 1. Introduction

Advances in hygiene coupled with progress in modern medicine have ensured improved quality of life in the 21st century. While modern medicine has identified new methods to treat and control contracted infections, the alarming increase in the number of new drug resistant pathogen strains pose a significant challenge. Transfer of such pathogens often takes place via physical contact and result in infections which are difficult to treat [1]. Deterring transfer of such pathogens from surfaces can reduce the probability of infection transmission. While several approaches including antimicrobial surface coatings, cleaning substrates with antimicrobial agents, UV-irradiation etc., have been evaluated to disinfect pathogens on surfaces, proper hand hygiene and skin disinfection still remains the most practical method to prevent infection transmission [2]. Disinfection is defined as 'a process that eliminates many or all pathogenic micro-organisms on inanimate objects with the exception of bacterial endospores' [3]. Although, significant efforts have been devoted to the discovery of novel antimicrobial chemicals, antimicrobial materials and other alternate strategies for infection prevention; most methods for disinfection involve microbial inactivation by killing them [4–8]. Increased reliance on germ kill actives however, raises concerns about development of antimicrobial resistance to such actives. The FDA's recent decision to outright ban 19 actives used in antimicrobial soaps, reflects this concern and emphasizes the need to reduce excessive use of common antimicrobial actives like triclosan and triclocarban [9].

The current study investigates alternate approaches for substrate disinfection within the context of hand washing. Hand hygiene and skin disinfection are a subset of substrate disinfection, with defined set of boundary conditions including very small exposure times coupled with limits imposed by cytotoxicity and biocompatibility of disinfecting agents. Common disinfection approaches have involved the use of antimicrobial soaps and hand sanitizers to kill microbes to significantly reduce the odds of infection transfer. In contrast, the current study investigates a removal-based disinfection approach using engineered particles. Silica particles were modified with cationic polymers for broad spectrum interaction with bacterial cells to test the hypothesis of particle enabled stripping of bacteria from surfaces. The hypothesis 'applied shear force following strong bacteria – particle binding would enable removal of microbes from the underlying substrate' was tested. Additionally, particle design parameters were identified to improve probability of microbial removal under conditions related to hand washing. In this regard, effect of particle charge, size, velocity, and concentration were investigated.

## 2. Experimental section

### 2.1. Materials

Silica (0.4–0.6 μm, 2 μm, 10–14 μm and 150–250 μm), Tween80® and Polyethylenimine (PEI - $M_n$10,000) were obtained from Sigma-Aldrich (St. Louis, MO, USA); Silica (200 nm) was obtained from Geltech, Inc. (Alachua, FL, USA). Silica (40–63 μm), dehydrated media, ampicillin sodium salt and neutralizer broth (D/E broth) were obtained from Thermo Fisher Scientific. Artificial skin substrate (VITRO-SKIN®) was purchased from IMS Inc. (Portland, ME, USA). *Escherichia coli* (ATCC 25922GFP) was obtained from American Type Culture Collection (ATCC). Propagation of culture was done by growing *E. coli* in trypticase soy broth (TSB) supplemented with 100 μg/ml ampicillin at 37 °C, and harvested in log phase at $OD_{600}$ of 0.3–0.4 by centrifugation at 3200*g* for 15 min. Cell concentration was adjusted to $10^9$ CFU/ml ($OD_{600}$ 0.5) using water for further use.

### 2.2. Particle modification and characterization

Silica particles (1 g) were etched with 1 M HCl for 60 min and washed thrice to hydroxylate the silica surface and to remove organic contaminants. Subsequently, PEI (10 kDa) was adsorbed onto the particles via overnight functionalization (particles stirred in a flask with polymer for 17 h) with 40 ml 2 wt.% PEI at pH 10.5 in a rotisserie. Particles were harvested via centrifugation at 10,000 rpm (9000–17000*g*) for 30 min and washed thrice with deionized (DI) water to remove loosely adsorbed PEI followed by lyophilization (Labonco freeze drier) overnight for storage. Particle suspensions for bacterial removal experiments were prepared using 1 wt.% of modified particles (silica coated with PEI) or unmodified particles (unmodified silica) in 0.5% Tween 80 unless otherwise noted.

Following functionalization, modified and control (unmodified) particles were characterized for zeta potential using Brookhaven ZetaPlus, and for particle size using Coulter LS13320. Zeta potential measurements were performed at 10 mM KCl and pH 8.0–8.5. pH for zeta potential characterization was adjusted using 10 mM NaOH or 10 mM HCl, as necessary.

### 2.3. Substrate preparation and characterization

VITRO-SKIN® is a commercially available artificial skin substrate coated with collagen, gelatin and silica particles to mimic physicochemical properties of natural human skin including pH, topography, and ionic strength. The substrate was prepared by hydrating a 1.5 cm × 1.5 cm patch, overnight, using glycerol: water (15:85) binary mixture in a humidity chamber, as suggested by the manufacturer. Zeta potential of the substrate was measured using Paar Physica Electro Kinetic Analyzer at 10 mM KCl and pH 6.7. Additionally, Contact angle measurements with water ($\sigma_L^P = 46.4$, $\sigma_L^D = 26.4$), ethylene glycol ($\sigma_L^P = 21.3$, $\sigma_L^D = 26.4$) and diiodomethane ($\sigma_L^P = 1.8$, $\sigma_L^D = 49.0$) were carried out using the sessile drop method within 60 s of deposition, and were used to estimate critical surface energy of the artificial skin substrate using the Owens Wendt model [10].

### 2.4. Characterization of bacteria

The *E. coli* strain employed in this study (ATCC 25922GFP) was characterized for surface energy using a light scattering technique detailed by Zhang et al. [11]. Briefly, *E. coli* cells at set concentration were suspended in ethanol: water binary mixtures of varying surface tension, and vortexed for 30 s at 1500 rpm before leaving them undisturbed for 20 min. Next, suspensions were centrifuged at 43*g* (RCF) for 45 s and supernatants were measured for optical density at 600 nm. Suspension with the highest optical density (OD 600 nm) was determined as closest to the surface energy value of the bacterial cell. Zeta potential measurements for bacterial cells were conducted according to the protocol outlined previously for particles.

### 2.5. Particle - bacteria interaction and removal assays

Bacterial removal from substrate was assessed using a custom-built protocol, based on multiple ASTM standards (ASTM E1197, ASTM E2897, ASTM E1837). Specifically, 10 μl suspension of *E. coli* containing $10^7$ cells was first spread over 1 $cm^2$ area of hydrated skin substrate and air dried at room temperature for 30 min. Next, inoculated skin substrates were exposed to modified or control particles in a 2 ml polypropylene microfuge tube, and vortexed for 30 s at 1500 RPM with 1 s pulse duration. Subsequently, substrates were rinsed with DI water (10 s vortex with 1 s pulse, 1500 RPM) to detach loosely bound particles from the