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# Insight into membrane selectivity of linear and branched polyethylenimines and their potential as biocides for advanced wound dressings

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ABSTRACT

We report here structure-property relationship between linear and branched polyethylene imines by examining their antimicrobial activities against wide range of pathogens. Both the polymers target the cytoplasmic membrane of bacteria and yeasts, eliciting rapid microbicidal properties. Using multiscale molecular dynamic simulations, we showed that, in both fully or partially protonated forms LPEI discriminates between mammalian and bacterial model membranes whereas BPEI lacks selectivity for both the model membranes. Simulation results suggest that LPEI forms weak complex with the zwitterionic lipids whereas the side chain amino groups of BPEI sequester the zwitterionic lipids by forming tight complex. Consistent with these observations, label-free cell impedance measurements, cell viability assays and high content analysis indicate that BPEI is cytotoxic to human epithelial and fibroblasts cells. Crosslinking of BPEI onto electrospun gelatin mats attenuate the cytotoxicity for fibroblasts while retaining the antimicrobial activity against Gram-positive and yeasts strains. PEI crosslinked gelatin mats elicit bactericidal activity by contact-mediated killing and durable to leaching for 7 days. The potent antimicrobial activity combined with enhanced selectivity of the crosslinked ES gelatin mats would expand the arsenel of biocides in the management of superficial skin infections. The contact-mediated microbicidal properties may avert antimicrobial resistance and expand the diversity of applications to prevent microbial contamination.

**Statement of Significance**

Current commercially available advanced wound dressings are either impregnated with metallic silver or silver salts which have side effects or may not avert antimicrobial resistance. In this article, we have used multidisciplinary approach comprising of computational, chemical and biological methods to understand the antimicrobial properties and biocompatibility of linear (LPEI) and branched (BPEI) polyethylenimines. We then applied this knowledge to develop dual purpose wound dressings containing these polymers, which encourages healing while maintain antimicrobial activity. In addition, the approach can be

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expanded to rationalize the antimicrobial vs. cytotoxicity of other cationic polymers and the method of crosslinking would enhance their potentials as biocides for advanced materials.


## 1. Introduction

In recent years, several antimicrobial surveillance programs have highlighted the evolution of drug-resistant pathogens against conventional antibiotics and emphasized the need for strategies that can combat antibiotic-resistant pathogens [1,2]. In the United States alone, hospital acquired infections (HAI) account for 100,000 deaths and >$20 billion health care costs every year [3–5]. Since about 80% HAI are attributed to medical devices, there is a perceived interest by medical device manufacturers for developing antimicrobial-embedded materials. In particular, there is an increasing demand for advanced wound dressings for acute wounds from burns or surgery and chronic wounds such as diabetic/venous ulcers [6–8]. For ageing, diabetic and immunocompromised populations, infections caused by antibiotic resistant pathogens in chronic wounds present a greater challenge as they delay the healing process and increase duration of hospital days and nursing costs [6,9,10]. The polymicrobial nature of chronic wound infections reduces the appropriate choice of therapeutic options to manage these infections [11]. The most common aetiological agents responsible for burns or chronic wound infections are the Gram-positive cocci (*Staphylococcus* and *Streptococcus* species) and to a lesser extent the Gram-negative *Enterobacteriaceae* and *Pseudomonas* species [12,13].

Current treatment options to overcome wound infections include topical applications of antibiotics or cleaning with antiseptic agents as well as the use of advanced wound dressings which contain topical antimicrobial agents [14,15]. The advanced wound dressings are impregnated with honey, silver, silver sulfadiazine, cadexomer iodine and cationic polymers to prevent local infections in chronic wounds [16–18]. However, concerns have been raised with respect to their indiscriminate and prolonged applications as well as antimicrobial resistance to silver/silver-based antimicrobials [19]. Molecular entities that selectively target the cytoplasmic membrane of microbes are useful alternative for averting antibiotic resistance [20]. A number of cationic antimicrobial peptides, synthetic polymers or synthetic derivatives of natural products have been reported as potent membrane-active antimicrobial agents [21–27]. Polyethylenimines (PEIs) belong to the class of synthetic cationic polymers with excellent transfection efficiency for gene delivery and antiviral properties [28,29]. A number of quaternized derivatives of PEIs in the form of nanoparticles or coatings have been shown to elicit potent antimicrobial activity against Gram-positive and Gram-negative pathogens [30–33]. However, there exist only a limited number of studies that have evaluated the antimicrobial and cytotoxicity of PEIs in their unmodified native forms. For example, recent studies have shown that linear or branched PEIs displayed potent antimicrobial activity against *S. aureus* and their mammalian cell cytotoxicity depend on the polymer molecular weight and exposure time; yet the mechanism of antimicrobial action remains unclear [34,35]. The use of PEIs as biocides is attractive because PEI-based hydrogels have been approved by the US FDA as surgical sealants and PEI-coated medical devices are under clinical trials for extracorporeal blood purification therapies [36,37].

Therefore, in this article, we investigated the antimicrobial properties of linear and branched PEIs (LPEI/BPEI) against a panel of Gram-negative, Gram-positive and yeasts pathogens, which include clinical isolates and antibiotic-resistant strains. Using multiscale molecular dynamic (MD) simulations, we demonstrate the differences in selectivity of LPEI and BPEI between bacterial and mammalian model membranes. The simulation results are corroborated by biophysical methods, label-free cell impedance and high content cytotoxicity analyses. Finally, we demonstrate that crosslinking of LPEI/BPEI on to an electrospun (ES) gelatin matrix can attenuate the cytotoxicity with retention of antimicrobial activity against Gram-positive and yeast pathogens. The polymer loaded mats displayed bactericidal activity on contact, elicit rapid bactericidal activity and durable to leaching for 7 days. The development of contact-mediated non-leachable antimicrobial nanofibre mats have the potential to avert the evolution of antimicrobial resistance, attenuate the mammalian cell cytotoxicity associated with the biocides and hold promise for the preparation of protective apparels, food packaging materials as well as effective and long-term management of burn wounds and chronic ulcers.

## 2. Experimental section

### 2.1. Microorganisms

A wide variety of Gram-positive, Gram-negative and yeasts pathogens were included in this study. The antimicrobial efficacy of the polymers were evaluated against both the reference strains from American Type Culture Collection (ATCC) and clinical isolates to infer if there is any differences in the activity (Tables S1–S3). A detailed description of the antimicrobial testing, disc diffusion, time-kill kinetics and membrane depolarization assays are provided as Supporting Information. Linear ($M_n \sim 20{,}000$, catalogue no. 764965) and branched ($M_n \sim 10{,}000$, catalogue no. 408727) polyethylene imine hydrochloride were purchased from Sigma-Aldrich (S) Pte Ltd., Singapore.

### 2.2. Molecular dynamics simulations

Two membrane systems were created [38–40], a POPC/Cholesterol model with a ratio of 10:8, and a POPE/POPG model with a ratio of 4:1. All simulations were run in the AMBER package [41,42]. The AMBER lipid14 force field [43] was chosen to describe the membrane atoms, and the generalised amber force field (GAFF) [44] was used to describe the polymers (LPEI and BPEI). Parameters for a single polymer unit were generated from quantum mechanical geometry optimisations of polymers five units in size, at HF/6-31G* level of theory and restrained electrostatic potential (RESP) partial charges obtained in NWChem [45]. LPEI of the desired length was created from a linear combination of these units, whereas the BPEI model was built from a more complex assembly of these PEI units. For LPEI models, a length of 20 monomer units was chosen, and for BPEI models, a length of 3 monomer units was chosen (corresponding to a backbone length of 19 LPEI monomer units). More details on simulations are provided in Supporting Information.

### 2.3. Real-time label-free monitoring of mammalian cell viability by xCELLigence system

Human telomerase immortalized corneal epithelial (hTCEpi) cells, a kind gift from Prof. Winston Kao (University of Cincinnati, Ohio, USA), were cultured in serum-/calcium-free keratinocyte culture media (DermaLife, Lifeline Cell Technology, MD, USA)

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