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

Fox, Stephen John; Fazil, Mobashar Hussain Urf Turabe; Dhand, Chetna; Venkatesh, Mayandi; Goh, Eunice Tze Leng; Harini, Sriram; Eugene, Christo; Lim, Rayne Rui; Ramakrishna, Seeram; Chaurasia, Shyam Sunder; Beuerman, Roger W.; Verma, Chandra Shekhar; Verma, Navin Kumar; Loh, Xian Jun; Lakshminarayanan, Rajamani

2016

Fox, S. J., Fazil, M. H. U. T., Dhand, C., Venkatesh, M., Goh, E. T. L., Harini, S., et al. (2016). Insight into membrane selectivity of linear and branched polyethylenimines and their potential as biocides for advanced wound dressings. *Acta Biomaterialia*, 37, 155-164.

<https://hdl.handle.net/10356/81965>

<https://doi.org/10.1016/j.actbio.2016.04.015>

© 2016 Acta Materialia Inc. (published by Elsevier Ltd.). This is the author created version of a work that has been peer reviewed and accepted for publication in *Acta Biomaterialia*, published by Elsevier Ltd. on behalf of Acta Materialia Inc. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [<http://dx.doi.org/10.1016/j.actbio.2016.04.015>].

Insight into Membrane Selectivity of Linear and Branched Polyethylenimines and Their Potential as Biocides for Advanced Wound Dressings

Stephen John Fox^{1,#}, Mobashar Hussain Urf Turabe Fazil^{2,#}, Chetna Dhand^{3,#}, Mayandi Venkatesh³, Eunice Tze Leng Goh³, Sriram Harini³, Christo Eugene³, Rayne Rui Lim⁴, Seeram Ramakrishna⁵, Shyam Sunder Chaurasia⁴, Roger W Beuerman^{3,6}, Chandra Shekhar Verma^{1,7,8,*}, Navin Kumar Verma^{2*}, Xian Jun Loh^{9*} and Rajamani Lakshminarayanan^{3,6*}

¹ Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR), Singapore 138671. ² Lee Kong Chian School of Medicine, Nanyang Technological University, Experimental Medicine Building, Singapore 636921. ³ Anti-Infectives Research Group, Singapore Eye Research Institute, Singapore 169856. ⁴ Ocular Immunology and Angiogenesis Lab, Department of Veterinary Medicine & Surgery, University of Missouri, Columbia, USA. ⁵ Department of Mechanical Engineering, National University of Singapore, Singapore 117576. ⁶ SRP in Neuroscience and Behavioral Disorders, Duke-NUS Graduate Medical School, Singapore 169857. ⁷ School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551. ⁸ Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543. ⁹ Institute of Materials Research & Engineering, A*STAR, 2 Fusionopolis Way, Innovis, #08-03, Singapore 138634.

[#]These authors contributed equally to this work.

Keywords: molecular dynamics, membrane selectivity, polyethylenimine, electrospinning, contact-mediated antibacterials

***Corresponding Authors**

Dr. Xian Jun Loh

2 Fusionopolis Way, Innovis, #08-03, Singapore 138634

Email: lohxj@imre.a-star.edu.sg

Tel: +65-64168932

Dr. N. K. Verma

Email: nkverma@ntu.edu.sg

Dr. R. Lakshminarayanan

Email: lakshminarayanan.rajamani@seri.com.sg

Dr. Chandra Shekhar Verma

Email: chandra@bii.a-star.edu.sg

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 arsenal 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 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.

Graphical Abstract

MD simulations unveil the differences in interactions between linear (LPEI) and branched (BPEI) polyethylenimines with model membranes. Experimental results support the simulations data that the lack of selectivity in BPEI is due to strong electrostatic and non-polar interactions with the lipid head groups and provides guidance to improve the selectivity of the polymers. By crosslinking the side chain groups we can attenuate the cytotoxicity of the polymer without losing antimicrobial activity.

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.^[21-25] Polyethylenimines (PEIs) belong to the class of synthetic cationic polymers with excellent transfection efficiency for gene delivery and antiviral properties.^[26,27] 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.^[28-31] 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.^[32,33] 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.^[34,35]

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 (**Table 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,^[36-38] 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.^[39,40] The AMBER lipid14 force field^[41] was chosen to describe the membrane atoms, and the generalised amber force field (GAFF)^[42] 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.^[43] 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) supplemented with 6 mM L-glutamine, 0.4% extract PTM, 1.0 μ M epinephrine, 0.5 ng/ml recombinant human (h)TGF α , 100 ng/ml hydrocortisone hemisuccinate, 5 μ g/ml recombinant human (h)Insulin, 5 μ g/ml apo-transferrin and maintained at 37°C in 5% CO₂. The xCELLigence system (ACEA, CA, USA) was used to determine hTCEpi cell proliferation and cell death in the presence of the polymers in real-time. Electronic impedance in the form of cell index (CI) was derived corresponding to the relative density and adherence strength of cells in each well. The hTCEpi cells (30×10^3 cells per well) were seeded on an E-plate 96. Confluency of cells in each well was determined according to CI values. At 70-80% confluency, culture medium was removed and replaced with hTCEpi media with indicated concentrations of polymers (0.125 – 1 mg/ml) in triplicates. Tobramycin (2.5 mg/ml), 10 mM PBS (pH 7.0) and 1% Triton-X100 served as controls. Culture was maintained for 24 h post addition with no media change in the xCelligence RTCA SP Station that was kept in an incubator at 37 °C with 5% CO₂. CI values were automatically calculated from the instrument after normalization (after addition of polymers/antibiotics) and compared with the control groups.

2.4 Electrospinning of PEI-loaded gelatin mats and crosslinking with dopamine: Type A porcine gelatin in trifluoroethanol (10% w/v) was mixed with dopamine hydrochloride (2% w/w of gelatin) and varying concentration of LPEI/BPEI (1%-10% w/w of gelatin) for overnight at room temperature. The dope solution was transferred into a 5 ml plastic syringe having 0.5 mm stainless steel needle and electrospun at room temperature at a relative humidity of 50%. The electrospinning parameters are as follows: applied voltage 11-14 kV, flow rate 1 ml/h and distance between needle tip and collector 12 cm. For morphological observations and cytotoxicity studies, the fibres were electrospun on a 10 mm microscopy coverslips placed on top of the collector aluminium foil. The collected electrospun nanofibers were vacuum dried for 48 h to remove any residual solvent present. For crosslinking, the as-

spun mats were placed in a closed desiccator along with a 5 g of $(\text{NH}_4)_2\text{CO}_3$ powder kept separately in an open vial for 24 h. The slow decomposition of $(\text{NH}_4)_2\text{CO}_3$ generates ammoniacal environment and induce the oxidative polymerization of dopamine.

2.5 High Content Screening and Analysis: To investigate the effect of polymers on various morphological parameters of cultured human dermal fibroblasts (hDF), we performed high content analysis (HCA), using an optimized and previously reported protocol as described.^[44] Briefly, hDF cells were seeded in 96-well plates (2×10^3 cells/well) and incubated for 24 h at 37°C and 5% CO_2 . Cells were then treated with various concentrations of polymers (LPEI or BPEI; 62.5, 125, 250, 500, 1000, or 2000 $\mu\text{g}/\text{ml}$ in triplicates) and further incubated for 24 h. After the treatment, cells were washed with PBS and fixed in 3% paraformaldehyde. Adherent cells were fluorescently stained with anti- α -tubulin-FITC and Alexa Fluor 569 phalloidin to visualize cellular morphologies and Hoechst to visualize the nuclei. Plates were scanned (12 randomly selected fields/well) using an automated microscope IN Cell Analyzer 2200 (GE Healthcare). Quantitative estimations of the acquired images were performed with IN Cell Investigator software using multi-parameter cytotoxicity bio-application module (GE Healthcare).

2.6 Statistical analysis: For statistical analysis, the data were analyzed by using GraphPad Prism 6.0 software. All the experimental data was expressed in mean $\pm$ standard deviations. The two-tailed Students t-test and one-way analysis of variance with Newman-Keuls post-hoc analysis were used for determining the significance of differences. The data with $P < 0.05$ was considered significance.

3. Results and Discussion

3.1 Antimicrobial properties of LPEI and BPEI

The antimicrobial properties of PEIs were examined against a panel of Gram-negative, Gram-positive and yeast pathogens in accordance with the Clinical and Laboratory Standard

document M100-S18 (**Table S1 – S3**). Among the various Gram-negative bacteria (GNB) examined, both the polymers displayed potent antimicrobial activity against *P. aeruginosa* only and the other GNBs required >2-4 fold higher concentrations for complete inhibition (**Table S1**). Both the polymers displayed superior antimicrobial activity against antibiotic-resistant clinical isolates when compared to tobramycin, a broad spectrum polycationic aminoglycoside antibiotics that is used widely for systemic and topical pseudomonal infections (**Table 1**). Similarly, among the various Gram-positive bacteria screened, *Staphylococcus* were more susceptible than *Enterococcus* or *Bacillus* species (**Table S2**). For both the polymers, the effective concentration for inhibiting *S. aureus* is 2-4 times lower than required for inhibiting *P. aeruginosa*. The presence of additional lipopolysaccharides membrane in GNBs acts as the permeability barrier, thus reducing the susceptibility against the positively charged PEIs when compared to Gram-positive bacteria which lack similar cell wall architecture.^[45] The antimicrobial effect of polymers was also remarkable against dimorphic pathogens *C. albicans* (**Table 1** and **S3**).

<<<<<<**Table 1**>>>>>>

3.2 Interaction of LPEI and BPEI with model membranes

To resolve the conflicting reports in the literature regarding the membrane targeting antimicrobial action of PEIs and to infer their selectivity^[32,33] for the prokaryotic cytoplasmic membrane over eukaryotic membrane, we performed multiscale MD simulations. For mammalian model membranes, a bilayer composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and cholesterol (POPC/Cho) was used whereas 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) in 4:1 ratio (POPE/PG) served as mimics of bacterial inner membrane.^[46] Molecular dynamics simulations were carried out for 300 ns at 310 K in the NpT-ensemble. Simulating the interaction of polymers with model membranes which mimic the composition of mammalian (POPC/Cho) and bacterial (POPE/PG) membranes would

provide atomistic details of the polymer-lipid interactions. The presence of cholesterol in the model membrane is important since the plasma membrane of mammalian cells contain 30 - 50 mol% cholesterol and a high content of cholesterol is associated with the formation of lipid rafts.^[47-49] The changes in molar ratio of cholesterol/phospholipid affect a number of membrane properties such as membrane fluidity, phase behavior, barrier properties, transport functions and conformation of membrane proteins.^[50-52]

The simulations of the polymers interacting with the two model membranes are shown in **Figure 1**. In the fully protonated form, LPEI polymers were unstable in the POPC/Cho membrane (*Supporting Movie 1*) and rapidly ejected (<10 ns) from the bilayer. This suggested that at low pH, LPEI did not engage in stable interactions with the zwitterionic membrane lipids. In contrast, the polymer formed a stable pore in the anionic POPE/PG bilayer (**Figure 1a**; *Supporting Movie 2*); the negatively charged PG head groups were selectively sequestered by LPEI molecules (**Figure 1b**). The pore region was filled with water molecules which also formed hydrogen bonding networks with the backbone imine, thus stabilising the LPEI-PG complex. However, BPEI formed more stable pores in both POPC/Cho and POPE/PG bilayers during the entire simulations as shown in **Figure 1c** and **1d**, respectively. Analysis of the simulations data suggest that, the average P-N distances in the head groups of the zwitterionic lipids match the average distance between the protonated amines ($-\text{NH}_2^+(\text{CH}_2)_2\text{NH}_2^+$) of the backbone LPEI (*Figure S1* and *Table S4*). This means that in the fully protonated form, cationic charges from the polymer and the trimethylammonium present in the head group of zwitterionic lipids, will have strong electrostatic repulsion. With respect to the zwitterionic POPC and POPE groups, the cationic amino groups present in the branches of BPEI allow for interaction with PO_4^{2-} whilst keeping the rest of the backbone away from the cationic $-\text{N}(\text{Me})_3^+$ or $-\text{NH}_3^+$ groups of POPC and POPE, respectively.

To discern if this difference was the cause for selectivity, we generated alternating charged/neutral polymer models and inserted into the two membranes (referred to as Alt-LPEI and Alt-BPEI). The uncharged nitrogen atoms may act as a Lewis base and form stable interactions with the cationic groups present in the zwitterionic head groups POPC and POPE. A stable complex of both partially protonated polymers embedded inside the POPC/Cho bilayer was observed (**Figure 1e-h**). When compared to fully protonated LPEI, Alt-LPEI forms additional, yet weak interactions with the POPC/Chol membrane, leading to the formation of a small pore (*Supporting Movie 3*).

To obtain more insight into the degree of bilayer disruption by LPEI and BPEI in various charged states, we estimated the amount of water molecules present across the membrane bilayers. The disruption of the membrane and the size of pores can be seen with the increase in number of water molecules present across the membrane bilayer. The water count was taken between the oxygens of the lipid chains averaged over the last 50 ns of the simulations. As can be seen from **Table S5**, BPEI confers the maximum disruption of the membranes independent of protonated or partially protonated forms. On the other hand, Alt-LPEI confers more perturbation to POPC/Cho bilayer and form a small pore than fully protonated LPEI, which was readily ejected from the bilayer interface (**Figure S2**).

3.3 In vitro evaluation of membrane selectivity of LPEI and BPEI

Results from MD simulations suggest that the absence of branching in the LPEI contributes to the greater selectivity in discriminating prokaryotic and eukaryotic cytoplasmic membranes than BPEI. To confirm these observations, we examined the antimicrobial properties and cytotoxicity of the polymers for epithelial and fibroblasts cells in detail. First, membrane depolarization assays demonstrate that addition of LPEI or BPEI to intact microbes resulted in rapid and substantial dissipation of membrane potential (**Figure S3-S5**). Bacterial viability assay performed in parallel with the membrane potential dissipation assay

further confirm that the loss of membrane potential is associated with bacterial lethality, thus demonstrating the membrane-targeting antimicrobial activity of the cationic polymers (**Figure S4c**).^[53-57]

Next, we examined the cytotoxicity of the polymers for the human telomerase-immortalized corneal epithelial cells (hTCEpi) by real time xCELLigence cell index (CI) impedance measurements. The CI values for hTCEpi cells (seeded at the density of 3×10^4 cells/well) growing in the wells of impedance E-plate increased with time and reached a plateau after 3 days (**Figure S6a**). Typical CI values after the addition of tobramycin or PBS buffer at this stage did not cause any change in CI values for over 24 h whereas the addition of Triton-X100 caused an instantaneous drop in the CI values due to detachment of the cells from the electrode surface, indicating spontaneous cytotoxic effects of the non-ionic detergent (**Figure S6a**). The dynamic changes in CI was monitored in real time upon addition of increasing concentrations of LPEI/BPEIs. Cells treated with LPEI at concentrations of 125 µg/ml or 250 µg/ml did not show any appreciable changes in CI values (**Figure 2a**). However, significant loss in hTCEpi cell attachment (indicated by decrease in CI values) was observed when the concentration of LPEI exceeded 500 µg/ml. The loss of CI values was apparent 12 h post addition of LPEI indicating programmed cell death. However, for the BPEI an instantaneous decrease in the CI values was observed even at the lowest concentration (125 µg/ml) tested in this study, indicating rapid cell death (**Figure 2b**). Normalization of the CI values after the addition of controls/polymers indicated significant ($p < 0.0001$) cytotoxicity conferred by linear PEI above 500 µg/ml compared to positive controls (**Figure S6b**). For BPEI, substantial cytotoxicity was observed even at 125 µg/ml (**Figure S6c**). Thus, xCELLigence assay on epithelial cell cytotoxicity results confirm that LPEI and BPEI differ in their selectivity between prokaryotic and eukaryotic cells, validating the results derived from the MD simulations.

In vitro assessment of biocompatibility of PEIs for possible topical applications is essential to establish the therapeutic potential of these polymers. Previous studies have shown that mammalian cell cytotoxicity of PEIs depend upon the polymer molecular weight and exposure time.^[33] To obtain a better insight into possible LPEI/BPEI-induced mammalian toxicity, we used High Content Analysis (HCA), a high throughput quantitative data acquisition method for determining changes in cellular behaviour and morphotypes.^[58-60] For this study, human dermal fibroblasts (hDFs) cells were exposed to various concentrations of the polymers for 24 h, fluorescently stained with nuclear and cytoplasmic markers and analysed by using an automated INCell Analyzer 2200. When compared to the untreated control cells, no apparent changes in the gross morphology and adhesion was observed for the cells exposed to LPEI up to 500 µg/ml (**Figure 2c**). At this concentration, the overall shapes and average sizes of the cells and nuclei were within the normal variation range and there were no signs of significant cellular or nuclear abnormalities, membrane bound vesicles, or cell rupture. With increasing concentration of the polymer, cells with reduced size and lower number of stress fibre formation appeared. In contrast, cells treated with 62.5 µg/ml of BPEI displayed considerable reduction in size and complete disappearance of stress fibre formation (**Figure 2d**), indicating enhanced cytotoxicity of the polymer. Quantitative information on the polymer induced cell morphologies were analysed and the numerical values were converted into colour-coded heat maps by INCell Investigator (**Figure 2e**). The representative parameters include cell count, cell intensity, nuclear intensity and cell form factor. For LPEI, the nuclear area and the cell count which are direct measure of cell cytotoxicity, changed in a concentration-dependent manner whereas minor changes were observed for cell form factor and cell intensity. However, for the cells exposed to BPEI all the four parameters changed dramatically compared to untreated cells, indicating heightened cytotoxicity of the polymer. To validate the HCA results, we determined the viability of human dermal fibroblasts by MTS assay as well. As shown in **Figure 2f**, increasing the concentration of LPEI beyond 250

$\mu\text{g/mL}$ decreased the cell viability significantly ($P < 0.0001$) compared to control; whereas the presence of small amount of BPEI ($62.5 \mu\text{g/mL}$) caused substantial loss ($>80\%$) of cell viability (**Figure 2g**).

<<<<<<**Figure 2**>>>>>>

3.4 Polydopamine crosslinking onto electrospun gelatin attenuates cytotoxicity of PEIs

Crosslinking of PEIs has been shown to attenuate their cytotoxicity without affecting the transfection properties.^[61,62] Therefore, to expand the possible therapeutic utility of PEIs for topical wound dressings, gelatin mats containing various amounts of LPEI or BPEI were electrospun. The polymers were crosslinked to the ES gelatin nanofibres through the latent oxidative polymerization of dopamine. ES gelatin mats loaded with 5% LPEI or BPEI crosslinked with polydopamine retained the fibre morphology and contained a number of soldered junctions with reticulated structures (**Figure 3a and 3b**).

ATR-FTIR results confirmed the polydopamine crosslinking of LPEI/BPEI to the ES gelatin mats (**Figure S7**). When compared to pristine gelatin mats, substantial changes were observed in the mats containing LPEI/BPEI after polydopamine crosslinking. The appearance of a broad hump around 2110 cm^{-1} , assigned to the substituted imines ($\nu_{\text{substituted C=N}}$) confirmed the crosslinking of LPEI to dopamine and gelatin.^[63-65] The presence of an intense peak at 3494 cm^{-1} (assigned to the $\nu_{(\text{O-H/N-H})}$ of polydopamine) and a shoulder around 3278 cm^{-1} (assigned to the 2° amine groups of LPEI/BPEI) further indicated the presence of LPEI/BPEI within polydopamine crosslinked gelatin mats. The appearance of new peaks around $2862/2866$ and $2930/2921 \text{ cm}^{-1}$ in the LPEI/BPEI crosslinked mats further confirmed the formation of pDA structure.^[65] For BPEI crosslinked mats, in addition to the above observed bands, a substantial masking of the amide A band of gelatin was observed indicating strong interaction of the backbone amides with BPEI and polydopamine. In both LPEI/BPEI crosslinked mats, a

significant shift in the amide I ($\nu_{C=O}$) band was observed indicating that the cationic polymers induced conformational changes in gelatin nanofibres.

To ascertain if the crosslinking could attenuate the cytotoxicity, we examined the biocompatibility of polymer-loaded mats after exposure of hDFs for 24 h. LPEI-loaded mats displayed good biocompatibility as only a marginal decrease in the cell viability was observed even in mats that contained 10% LPEI (**Figure 3c**). For BPEI-loaded mats, significant loss of viability was observed as the concentration of the polymer exceeded 5%. Nevertheless, at 1% and 2% polymer loading more cell viability was observed, as compared to free polymer in solution. When compared to free polymer solution, crosslinking of side chain amino groups in BPEI could bury their accessibility for direct interaction with the zwitterionic lipids present in the mammalian cells, thus attenuating its cytotoxicity. These results confirm that crosslinking of linear or branched PEIs on ES gelatin mats can attenuate the cytotoxicity. Morphological analysis further confirmed that fibroblasts cultured on 2% LPEI or BPEI retained the elongated shape with a number of stress fibres, similar to the cells grown on pristine gelatin mats (**Figure 3d-f**).

<<<<<<Figure 3>>>>>>>>

3.5 Antimicrobial properties of PEI-loaded crosslinked electrospun mats

We then determined the antimicrobial activity of crosslinked mats containing 2% LPEI by Kirby-Bauer disc diffusion assay. An extensive growth of microorganisms was observed on pristine electrospun gelatin mats (**Figure S8**). However, no clear zone of inhibition was observed around the crosslinked mats containing 2% LPEI against Gram-positive and yeast strains although no growth/colonies were detected on top and underneath of the mats (**Figure 4a**). These results suggest that the LPEI loaded mats displayed contact-mediated microbicidal activity. In contrast, the anti-*Pseudomonas* activity was completely lost upon crosslinking since significant growth was observed both on top and bottom of the mats as well as around the mats (data not shown). To ascertain the contact-mediated killing against Gram-positive

bacteria and yeasts, we evaluated the antimicrobial properties by the microbroth dilution method under the conditions that are conducive for microbial growth.^[66,67] Briefly, 1 cm² square strip of pristine gelatin mats as well as LPEI crosslinked mats were immersed in 2 ml of broth containing 2×10^7 CFU/ml of *S. aureus* ATCC 29737 and *MRSA* DB21595 or 2×10^5 CFU/ml of *C. albicans* ATCC 2091 and *C. albicans* DF 2672R strains. It should be noted that the amount of inoculum taken was 2log₁₀-fold higher than the observed bioburden in chronic wounds.^[68] After 24 h exposure, 100 µl aliquot of the solution was diluted log₁₀-fold serially and the viable bacterial/yeasts colonies were enumerated. A significant increase in the growth of both bacterial and yeast strains was observed in ES gelatin mats whereas mats containing LPEI conferred complete killing of Gram-positive and candida strains (**Figure 4b-e**). These results confirm the complete retention of microbicidal activity of LPEI crosslinked mats against pathogenic bacteria and yeasts.

The time-dependent killing capacity of LPEI crosslinked ES gelatin mats was examined against two Gram-positive strains as well under proliferating conditions (**Figure 4f and g**). The density of the bacterial culture increased from $10\text{--}20 \times 10^7$ CFU/ml at the beginning of the experiment to $>10^{13}$ CFU/ml in 24 h for both the Gram-positive strains. Similar increase in density was observed in the presence of pristine ES gelatin mats. When exposed to LPEI crosslinked mats, a significant decrease in bacterial burden was observed within the first few hours. In the presence of LPEI crosslinked mats $>3\log_{10}$ ($>99.9\%$) decrease in CFU/ml (compared to the initial inoculum of $\sim 10^7$ CFU/ml) was readily achieved in 2 h. No colonies were detected after 8 h exposure against the *MRSA* strains. The rapid bactericidal activity was significantly pronounced in the uncrosslinked mats. These results indicate that crosslinking of LPEI did not alter its antimicrobial properties although a delay in the kinetics of killing. Finally, we tested the durability of the mats by assessing the antimicrobial activity of LPEI crosslinked mats under dynamic conditions (**Figure 4h**). Briefly, the crosslinked mats were immersed in PBS (pH=7.0) for 1 day and 7 days with constant shaking. The mats were

removed, washed with deionised water for few times and immersed in bacterial inoculum. The antimicrobial activity was assessed by bacterial viability test as before.^[69,70] For a comparison, we have used a commercially available silver-based wound dressing mats, Aquacel[®] Ag. No colonies were detected after 1 day in both the LPEI crosslinked mats and Aquacel dressings, indicating excellent durability of the crosslinked mats. After 7 days, the crosslinked mats elicited 99.8% decrease (2.9 log₁₀ reduction) in bacterial viability whereas Aquacel[®] wound dressings decreased the viability by 99.98% (3.8 log₁₀ reduction). These results establish that the LPEI crosslinked mats possess comparable durability as that of commercial silver based wound dressings. It should be noted that the amount of biocide used in our experiments is half that of silver present in Aquacel wound dressings, yet both displayed similar antimicrobial activity under dynamic conditions.

<<<<<Figure 4>>>>>

4. Conclusion

In summary, using a multidisciplinary approach we have demonstrated the differences in selectivity of LPEI and BPEI for the mammalian and bacterial membranes. MD simulations results suggest that, in BPEI, the participation of sidechain interactions with the phosphate head groups of constituent bacterial/mammalian membrane lipids is responsible of the apparent lack of selectivity. In contrast, higher selectivity observed in LPEI is attributed to preferential sequestration of negatively charged lipids as well as destabilizing interaction between the backbone amines and quarternary nitrogen present in the zwitterionic lipids. In support of these observation, crosslinking of the sidechain amino groups present in BPEI on to an electrospun gelatin attenuated the heightened cytotoxicity observed for the free polymer in solution. Finally, we showed that the LPEI crosslinked mats displayed rapid bactericidal activity through contact-mediated killing and the mats were durable to washing for atleast 7

days. The potent antimicrobial activity against common pathogens of skin microflora combined with increased selectivity of the crosslinked ES gelatin mats could complement current strategies in the management of acute as well as chronic skin infections, avert antimicrobial resistance as no antimicrobial is released from the mats, and expand the diversity of applications wherein prevention of microbial contamination is required.

Acknowledgements

This research is supported by the Singapore National Research Foundation under its Translational and Clinical Research Flagship Program (NMRC/TCR/008-SERI/2013) and administered by the Singapore Ministry of Health's National Medical Research Council. RL thanks the funding support from National Medical Research Council's Co-operative Basic Research Grant (NMRC/CBRG/0048/2013). NKV acknowledges funding support from the NTU Lee Kong Chian School of Medicine Start-up grant (L0412130 and L0412290) and the Ministry of Education Singapore AcRF-Tier I (2014-T1-001-141) grants for high content screening of biocompatibility analysis. XJL acknowledges the A*STAR Personal Care Program fund as well as the Joint Council Office for support of his research.

References

- [1]. *The evolving threat of antimicrobial resistance: Options for action*, World Health Organization, Switzerland (2012).
- [2]. European Centre for Disease Prevention and Control (ECDC) and European Medicines Agency (EMA), ECDC/EMA Joint Technical Report-The bacterial challenge: time to react, http://www.emea.europa.eu/docs/en_GB/document_library/Report/2009/11/WC500008770.pdf, accessed: January 2012.
- [3]. Estimates of Healthcare-Associated Infections, <http://www.cdc.gov/ncidod/dhqp/hai.html> August 2010, accessed: August, (2010).
- [4]. Infectious Diseases Society of America (IDSA), B. Spellberg , M. Blaser , R. J. Guidos , H. W. Boucher , J. S. Bradley, B. I. Eisenstein, D. Gerding, R. Lynfield, L. B. Reller, J. Rex, D. Schwartz, E. Septimus, F. C. Tenover, D. N. Gilbert, Combating antimicrobial resistance: policy recommendations to save lives, *Clin. Infect. Dis.* 52 (2011) S397-428
- [5]. R. Douglas Scott II, The Direct Medical costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention, National Center for Preparedness, Detection, and Control of Infectious Diseases, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, USA (2009).
- [6]. C. K. Murray, Infections in burns, *J. Trauma.* 62 (2007) S73.
- [7]. L. I. Moura, A. M. Dias, E. Carvalho, H. C. de Sousa, Recent advances on the development of wound dressings for diabetic foot ulcer treatment—A review, *Acta Biomater.* 9 (2013) 7093-7114.
- [8]. P. G. Bowler, B. I. Duerden, D. G. Armstrong, Wound microbiology and associated approaches to wound management, *Clin. Microbiol. Rev.* 14 (2001) 244-269.
- [9]. L. K. Branski, A. Al-Mousawi, H. Rivero, M. G. Jeschke, A. P. Sanford, D. N. Herndon, Emerging infections in burns, *Surg. Infect.* 10 (2009) 389-397.
- [10]. R. L. Nichols, S. Florman, Clinical presentations of soft-tissue infections and surgical site infections, *Clin. Infect. Dis.* 33 (2001) S84-93.

- [11]. S. E. Dowd, R. D. Wolcott, Y. Sun, T. McKeehan, E. Smith, D. Rhoads, Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP), *PLoS One*, 3 (2008) e3326.
- [12]. B. A. Lipsky, J. A. Weigelt, V. Gupta, A. Killian, M. M. Peng, Skin, Soft Tissue, Bone, and Joint Infections in Hospitalized Patients: Epidemiology and Microbiological, Clinical, and Economic Outcomes, *Infect. Control. Hosp. Epidemiol.* 28 (2007) 1290-1298.
- [13]. P. Giordano, K. Weber, G. Gesin, J. Kubert, Skin and skin structure infections: treatment with newer generation fluoroquinolones, *Ther. Clin. Risk Manage.* 3 (2007) 309-317.
- [14]. T. Dai, Y. Y. Huang, S. K. Sharma, J. T. Hashmi, D. B. Kurup, M. R. Hamblin, Topical Antimicrobials for Burn Wound Infections, *Recent Pat. Anti-Infect. Drug Discovery.* 5 (2010) 124-151.
- [15]. B. A. Lipsky, C. Hoey, Topical antimicrobial therapy for treating chronic wounds, *Clin. Infect. Dis.* 49 (2009) 1541-1549.
- [16]. V. Kostenko, J. Lyczak, K. Turner, R. J. Martinuzzi, Impact of silver-containing wound dressings on bacterial biofilm viability and susceptibility to antibiotics during prolonged treatment, *Antimicrob. Agents Chemother.* 54 (2010) 5120-5131.
- [17]. R. M. Thorn, J. Greenman, A. Austin, An in vitro study of antimicrobial activity and efficacy of iodine-generating hydrogel dressings, *J. Wound Care.* 15 (2006) 305-310.
- [18]. W. R. Lee, K. M. Tobias, D. A. Bemis, B. W. Rohrbach, In vitro efficacy of a polyhexamethylene biguanide-impregnated gauze dressing against bacteria found in veterinary patients, *Vet. Surg.* 33 (2004) 404-411.
- [19]. *Best Practice Statement: The use of topical antiseptic/antimicrobial agents in wound management*, Wounds UK, Aberdeen (2010).

- [20]. J. G. Hurdle, A. J. O'Neill, I. Chopra, R. E. Lee, Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections, *Nat. Rev. Microbiol.* 9 (2011) 62-75.
- [21]. C. P. Gerba, Quaternary ammonium biocides: efficacy in application, *Appl. Environ. Microbiol.* 81 (2015) 464-469.
- [22]. S. Biswas, J. M. Brunel, J. C. Dubus, M. Reynaud-Gaubert, J. M. Rolain, Colistin: an update on the antibiotic of the 21st century, *Expert Rev. Anti-Infect. Ther.* 10 (2012) 917-934.
- [23]. R. M. Epand, R. F. Epand, P. B. Savage, Ceragenins (cationic steroid compounds), a novel class of antimicrobial agents, *Drug News Perspect.* 21 (2008) 307-311.
- [24]. F. Porcelli, B. A. Buck-Koehntop, S. Thennarasu, A. Ramamoorthy, G. Veglia, Structures of the dimeric and monomeric variants of magainin antimicrobial peptides (MSI-78 and MSI-594) in micelles and bilayers, determined by NMR spectroscopy, *Biochemistry* 45 (2006) 5793-5799.
- [25]. H. Zou, J. J. Koh, J. Li, S. Qiu, T.T. Aung, H. Lin, R. Lakshminarayanan, X. Dai, C. Tang, F. H. Lim, L. Zhou, A. L. Tan, C. Verma, D. T. Tan, H. S. Chan, P. Saraswathi, D. Cao, S. Liu, R. W. Beuerman, Design and synthesis of amphiphilic xanthone-based, membrane-targeting antimicrobials with improved membrane selectivity, *J. Med. Chem.* 56 (2013) 2359-2373.
- [26]. L. Parhamifar, A. K. Larsen, A. C. Hunter, T. L. Andresen, S. M. Moghimi, Polycation cytotoxicity: a delicate matter for nucleic acid therapy—focus on polyethylenimine, *Soft Matter* 6 (2010) 4001-4009.
- [27]. G. A. Spoden, K. Besold, S. Krauter, B. Plachter, N. Hanik, A. F. Kilbinger, C. Lambert, L. Florin, Polyethylenimine is a strong inhibitor of human papillomavirus and cytomegalovirus infection, *Antimicrob. Agents Chemother.* 56 (2012) 75-82.

- [28]. S. Beyth, D. Polak, C. Milgrom, E. I. Weiss, S. Matanis, N. Beyth, Antibacterial activity of bone cement containing quaternary ammonium polyethyleneimine nanoparticles, *J. Antimicrob. Chemother.* 69 (2014) 854-855.
- [29]. N. Beyth, I. Yudovin-Farber, M. Perez-Davidi, A. J. Domb, E. I. Weiss, Polyethyleneimine nanoparticles incorporated into resin composite cause cell death and trigger biofilm stress in vivo, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 22038-22043.
- [30]. A. M. Klibanov, Permanently microbicidal materials coatings, *J. Mater. Chem.* 17 (2007) 2479-2482.
- [31]. B. Gao, X. Zhang, Y. Zhu, Studies on the preparation and antibacterial properties of quaternized polyethyleneimine, *J. Biomater. Sci., Polym. Ed.* 18 (2007) 531-544.
- [32]. K. A. Gibney, I. Sovadinova, A. I. Lopez, M. Urban, Z. Ridgway, G. A. Caputo, K. Kuroda, Poly (ethylene imine)s as antimicrobial agents with selective activity, *Macromol. Biosci.* 2 (2012) 1279-1289.
- [33]. C. Wiegand, M. Bauer, U. C. Hippler, D. Fischer, Poly (ethyleneimines) in dermal applications: Biocompatibility and antimicrobial effects, *Int. J. Pharm.* 456 (2013) 165-174.
- [34]. Premarket approval application (PMA) for the Adherus® AutoSpray Dural Sealant, http://www.accessdata.fda.gov/cdrh_docs/pdf13/P130014a.pdf, (2013).
- [35]. The Effects of a Polyethyleneimine-coated Membrane (oXiris™) for Hemofiltration Versus Polymyxin B- Immobilized Fibre Column (Toraymyxin™) for Hemoperfusion on Endotoxin Activity and Inflammatory Conditions in Septic Shock- A Randomized Controlled Pilot Study (ENDoX-study), <https://clinicaltrials.gov/ct2/show/study/NCT01948778>, (2015).

- [36]. S. Jo, T. Kim, V. G. Iyer, W. Im, CHARMM-GUI: a web-based graphical user interface for CHARMM, *J. Comput. Chem.* 29 (2008) 1859-1865.
- [37]. E.L. Wu, X. Cheng, S. Jo, H. Rui, K.C. Song, E.M. Dávila-Contreras, Y. Qi, J. Lee, V. Monje-Galvan, R.M. Venable, J.B. Klauda, W. Im, CHARMM-GUI Membrane builder toward realistic biological membrane simulations. *J. Comput. Chem.* 35 (2014) 1997-2004.
- [38]. S. Jo, J.B. Lim, J.B. Klauda, W. Im, CHARMM-GUI Membrane builder for mixed bilayers and its application to yeast membranes. *Biophys. J.* 97 (2009) 9750-9758.
- [39]. D. A. Case, T. E. Cheatham, III, T. Darden, H. Gohlke, R. Luo, K. M. Merz, Jr., A. Onufriev, C. Simmerling, B. Wang and R. Woods. The amber biomolecular simulation programs, *J. Computat. Chem.* 26 (2005) 1668-1688.
- [40]. R. Salomon-Ferrer, D. A. Case, R. C. Walker, An overview of the amber simulation package, *WIREs Comput. Mol. Sci.* 3 (2013) 198-210.
- [41]. C. J. Dickson, B. D. Madej, Å. A. Skjevik, R. M. Betz, K. Teigen, I. R. Gould, and R. C. Walker, Lipid14: the amber lipid force field, *J. Chem. Theory Comput.* 10 (2014) 865-879.
- [42]. J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, Development and testing of a general amber force field, *J Comput. Chem.* 25 (2004) 1157–1174.
- [43]. M. Valiev, E.J. Bylaska, N. Govind, K. Kowalski, T.P. Straatsma, H.J.J. van Dam, D. Wang, J. Nieplocha, E. Apra, T.L. Windus, W.A. de Jong, NWChem: a comprehensive and scalable open-source solution for large scale molecular simulations, *Comput. Phys. Commun.* 181 (2010) 1477-1489.
- [44]. N. K. Verma, J. Conroy, P. E. Lyons, J. Coleman, M. P. O’Sullivan, H. Kornfeld, D. Kelleher, Autophagy induction by silver nanowires: A new aspect in the biocompatibility assessment of nanocomposite thin films, *Toxicol. Appl. Pharmacol.* 264 (2012) 451-461.

- [45]. A. D. Russel, Similarities and differences in the responses of microorganisms to biocides, *J. Antimicrob. Chemother.* 52 (2003) 750-763.
- [46]. K. Murzyn, T. Rog, M. Pasenkiewicz-Gierula, Phosphatidylethanolamine-phosphatidylglycerol bilayer as a model of the inner bacterial membrane, *Biophys. J.* 88 (2005) 1091-1103.
- [47]. P. Emmelot, The organization of the plasma membrane of mammalian cells: structure in relation to function, in: G.A. Jamieson, D.M. Robinson (Eds.), *Mammalian Cell Membranes*, Butterworths, London, 1977, pp. 1-54.
- [48]. R. B. Gennis, in: *Biomembranes: Molecular Structure and Function*, Springer, New York, 1989, pp. 1-35.
- [49]. E. London, D. A. Brown. Insolubility of lipids in triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). *Biochim Biophys Acta.* 1508 (2000) 182-95.
- [50]. M. Hao, S. Mukherjee, F. R. Maxfield. Cholesterol depletion induces large scale domain segregation in living cell membranes. *Proc Natl Acad Sci U S A.* 98 (2001) 13072-7.
- [51]. H. Ohvo-Rekilä, B. Ramstedt, P. Leppimäki, J. P. Slotte. Cholesterol interactions with phospholipids in membranes. *Prog Lipid Res.* 41 (2002) 66-97.
- [52]. D. A. Brown, E. London. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J Biol Chem.* 275 (2000) 17221-4.
- [53]. J. A. Silverman, N. G. Perlmutter, H. M. Shapiro, Correlation of daptomycin bactericidal activity and membrane depolarization in *Staphylococcus aureus*, *Antimicrob. Agents Chemother.* 47 (2003) 2538-2544.
- [54]. I. S. Radzishhevsky, S. Rotem, D. Bourdetsky, S. Navon-Venezia, Y. Carmeli, A. Mor, Improved antimicrobial peptides based on acyl-lysine oligomers, *Nat. Biotechnol.* 25 (2007) 657-659.

- [55]. R. Lakshminarayanan, S. Liu, J. Li, M. Nandhakumar, T. T. Aung, E. Goh, J. Y. Chang, P. Saraswathi, C. Tang, S. R. Safie, L. Y. Lin, H. Riezman, Z. Lei, C. S. Verma, R. W. Beuerman, Synthetic multivalent antifungal peptides effective against fungi *PLoS One*. 9 (2014) e87730.
- [56]. C. L. Friedrich, D. Moyles, T. J. Beveridge, R. E. Hancock, Antibacterial action of structurally diverse cationic peptides on gram-positive bacteria, *Antimicrob. Agents Chemother.* 44 (2000) 2086-2092.
- [57]. M. Cheng, J. X. Huang, S. Ramu, M. S. Butler, M. A. Cooper, Ramoplanin at bactericidal concentrations induces bacterial membrane depolarization in *Staphylococcus aureus*, *Antimicrob. Agents Chemother.* 58 (2014) 6819-6827.
- [58]. F. Byrne, A. Prina-Mello, A. Whelan, B. M. Mohamed, A. Davies, Y. Gun ko, J. M. D. Coey, Y. Volkov, High content analysis of the biocompatibility of nickel nanowires, *J. Magn. Mater.* 321 (2009) 1341-1345.
- [59]. D. Movia, A. Prina-Mello, Y. Volkov, S. Giordani, Determination of spiropyran cytotoxicity by high content screening and analysis for safe application in bionanosensing, *Chem. Res. Toxicol.* 23 (2010) 1459-1466.
- [60]. N. K. Verma, E. Moore, W. Blau, Y. Volkov, P. R. Babu, Cytotoxicity evaluation of nanoclays in human epithelial cell line A549 using high content screening and real-time impedance analysis, *J. Nanopart. Res.* 14 (2012) 1137.
- [61]. M. Breunig, U. Lungwitz, R. Liebl, A. Goepferich, Breaking up the correlation between efficacy and toxicity for nonviral gene delivery, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 14454-14459.
- [62]. P. Xu, G. K. Quick, Y. Yeo, Gene delivery through the use of a hyaluronate-associated intracellularly degradable crosslinked polyethyleneimine, *Biomaterials* 30 (2009) 5834-5843.

- [63]. C. Zhao, F. Zuo, Z. Liao, Z. Qin, S. Du, Z. Zhao, Mussel-Inspired One-Pot Synthesis of a Fluorescent and Water-Soluble Polydopamine–Polyethyleneimine Copolymer, *Macromol. Rapid Commun.* 36 (2015) 909-915.
- [64]. S. S. York, S. E. Boesch, R. A. Wheeler, R. Frech, Vibrational assignments for high molecular weight linear polyethylenimine (LPEI) based on monomeric and tetrameric model compounds, *Macromolecules* 36 (2003) 7348-7351.
- [65]. R. A. Zangmeister, T. A. Morris, M. J. Tarlov, Characterization of polydopamine thin films deposited at short times by autoxidation of dopamine, *Langmuir* 29 (2013) 8619-8628.
- [66]. Clinical and Laboratory Standard Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Information Supplement*, CLSI document M100-S18, Wayne, PA, Clinical and Laboratory Standard Institute, (2008).
- [67]. O. Tkachenko, A. Karas, Standardizing an in vitro procedure for the evaluation of the antimicrobial activity of wound dressings and the assessment of three wound dressings. *J. Antimicrob. Chemother.* 67 (2012) 1697-1700.
- [68]. T. Weed, C. Ratliff, D. B. Drake, Quantifying bacterial bioburden during negative pressure wound therapy: does the wound VAC enhance bacterial clearance? *Ann. Plast. Surg.* 52 (2004) 276-279.
- [69]. Z. Li, P. L. Chee, C. Owh, R. Lakshminarayanan and X. J. Loh, Safe and efficient membrane permeabilizing polymers based on PLLA for antibacterial applications, *RSC Advances*, DOI: 10.1039/C6RA04531F (2016).
- [70]. Q. Q. Dou, X. T. Fang, S. Jiang, P. L. Chee, T. C. Lee and X. J. Loh, Multi-functional fluorescent carbon dots with antibacterial and gene delivery properties, *RSC Advances*, 5 (2015) 46817-46822.

Figure Legends

Figure 1. MD simulations on membrane-PEI interactions. (a) LPEI with POPE/PG bilayers. (b) Atomistic interactions showing stable complex between the negatively charged PG and LPEI/BPEI and PG. A 300 ns snapshots showing the interaction of BPEI with Zwitterionic POPC/Cho (c) and anionic POPE/PG (d) bilayers. Interaction of alternate charged BPEI with POPC/Cho (e) and anionic POPE/PG (f) bilayers. Interaction of alternate charge LPEI with POPC/Cho (g) and anionic POPE/PG (h) bilayers. The PEI molecules are depicted in a space filling representation, whilst the water molecules are show as red beads and the membrane bilayer is shown as a surface. The lipid tails are cyan, the cholesterol molecules are sky blue, the PC groups are gold, the PE groups are yellow and the PG groups are green.

Figure 2. Cytotoxicity analyses of LPEI and BPEI for epithelial (hTCEpi) cells and human fibroblasts (hDFs). Dyamic monitoring of hTCEpi cell cytotoxicity of polymers using xCELLigence system. Typical cell impedance (CI) traces showing concentration-dependent changes in the CI values after the addition of (a) LPEI and (b) BPEI. High content imaging and analysis of hDFs exposed to various concentrations of LPEI (c) or BPEI (d) for 24 h. (e) Heat map tables indicating quantification of various parameters (number of adherrand cells, form factor, cell area and nuclear area) determined from the high content imaging. MTS assay showing hDFs viability after exposure to various concentrations of LPEI (f) and BPEI (g) for 24 h. The concentration of polymers is expressed in $\mu\text{g/ml}$. NT indicates no treatment and Noco indicates nocodazole (5 $\mu\text{g/ml}$). ** $P < 0.01$ and **** $P < 0.0001$ after Dunnett's test for multiple comparisons.

Figure 3. (a) Scanning electron micrographs of electrospun gelatin mats containing 5% (w/w) of (a) LPEI and (b) BPEI after crosslinking with polydopamine. Scale bar = 1 μm . (c) Biocompatibility of ES gelatin mats for hDFs assessed by MTS assay. Fluorescence microscopic images of hDFs cultured on pristine ES gelatin (d) and mats containing 2% LPEI (e) or BPEI (f) after 24 h exposure. The cells were fluorescently stained with anti tubulin-FITC (green), Alexa Fluor 569 phalloidin (red) and Hoechst (blue). Florescent microscopic images were acquired using 40 $\times$ objective lens.

Figure 4. (a) Photographs of the disc diffusion assay for ES gelatin mats containing 2% LPEI after crosslinking with polydopamine. The strains tested are indicated in the figure. Note that no clear zone of inhibition surrounding the ES mats containing PEI, suggesting contact-mediated killing. Microbicidal properties of 2% PEI loaded ES gelatin mats against (b) *S. aureus* ATCC 29737, (c) MRSA DB 21595, (d) *C. albicans* ATCC 2091 and (e) *C. albicans* DF 2672R. Time-kill kinetics of ES gelatin mats containing 2% LPEI after crosslinking with polydopamine against (f) *S. aureus* ATCC 29737 and (g) MRSA DB 21595 strains. For a comparison ES gelatin mats containing 2% LPEI before crosslinking with dopamine was also included in the figure. (h) Antimicrobial durability of the mats to leaching, tested against the MRSA DB21595 strains. For a comparison results from Aquacel[®] Ag mats is also shown.

Table 1. Antimicrobial properties of LPEI/BPEI against reference and drug-resistant pathogens. ND indicates not determined. The clinical isolates are indicated by asterisks.

Strains	Minimum Inhibitory Concentration (μ M) of		
	LPEI	BPEI	Tobramycin
<i>Pseudomonas aeruginosa</i> ATCC 27853	3.2	6.4	<1.1
<i>Pseudomonas aeruginosa</i> * 07DM023376 (eye)	3.2	3.2	<1.1
<i>Pseudomonas aeruginosa</i> 230DR4877/07 (sputum)*	3.2	3.2	>34.2
<i>Pseudomonas aeruginosa</i> DR18531*	1.6	1.6	>34.2
<i>Staphylococcus aureus</i> ATCC 29213*	1.6	1.6	2.1
MRSA DR42412 (sputum)*	1.6	1.6	>34.2
MRSA DB21455 (eye)*	1.6	1.6	>34.2
MRSA DB09808R (eye)*	1.6	1.6	>34.2
MRSA DB57964/04 (blood)*	1.6	1.6	>34.2
MRSA DB68004 (blood)*	1.6	1.6	>34.2
MRSA DB21595 (wound)*	1.6	1.6	>34.2
<i>C. albicans</i> ATCC 10231	0.8	1.6	ND
<i>C. albicans</i> DF2672R*	0.8	1.6	ND

Figure 1. MD simulations on membrane-PEI interactions. (a) LPEI with POPE/PG bilayers. (b) Atomistic interactions showing stable complex between the negatively charged PG and LPEI/BPEI and PG. A 300 ns snapshots showing the interaction of BPEI with Zwitterionic POPC/Cho (c) and anionic POPE/PG (d) bilayers. Interaction of alternate charged BPEI with POPC/Cho (e) and anionic POPE/PG (f) bilayers. Interaction of alternate charge LPEI with POPC/Cho (g) and anionic POPE/PG (h) bilayers. The PEI molecules are depicted in a space filling representation, whilst the water molecules are show as red beads and the membrane bilayer is shown as a surface. The lipid tails are cyan, the cholesterol molecules are sky blue, the PC groups are gold, the PE groups are yellow and the PG groups are green.

Figure 2. Cytotoxicity analyses of LPEI and BPEI for epithelial (hTCEpi) and fibroblasts (hDFs). Dynamic monitoring of hTCEpi cell cytotoxicity of polymers using xCELLigence system. Typical xCELLigence traces showing the concentration-dependent changes in the CI values after the addition of (a) LPEI and (b) BPEI. High content imaging and analysis of the human dermal cells exposed to various concentrations of LPEI (c) or BPEI (d) for 24 h. (e) Heat map tables indicating quantification of various parameters determined from the high content imaging. MTS assay showing the fibroblasts viability after exposure to various concentrations of LPEI(f) and BPEI (g) for 24 h. The concentration of polymers is expressed in µg/ml. NT indicates no treatment and Noco indicates nocodazole (5 mg/ml). ** $P < 0.01$ and **** $P < 0.0001$ after Dunnett's test for multiple comparisons.

Figure 3. (a) Scanning electron micrographs of electrospun gelatin containing 5% (w/w) of (a) LPEI and (b) BPEI after crosslinking with polydopamine. Scale bar = 1 μ m. (c) Biocompatibility of ES gelatin mats for hDFs assessed by MTS assay. Fluorescence microscopic images of hDFs cultured on pristine ES gelatin (d) and mats containing 2% LPEI (e) or BPEI (f) after 24 h exposure. The cells were fluorescently stained with tubulin-FITC (green), Alexa Fluor 569 phalloidin (red) and Hoechst (blue). Florescent microscopic images were acquired using 40 $\times$ objective lens.

Figure 4. (a) Photographs of the disc diffusion assay for ES gelatin mats containing 2% LPEI after crosslinking with polydopamine. The strains tested are indicated in the figure. Note that no clear zone of inhibition surrounding the ES mats containing PEI, suggesting contact-mediated killing. Microbicidal properties of 2% PEI loaded ES gelatin mats against (b) *S. aureus* ATCC 29737, (c) MRSA DB 21595, (d) *C. albicans* ATCC 2091 and (e) *C. albicans* DF 2672R. Time-kill kinetics of ES gelatin mats containing 2% LPEI after crosslinking with

polydopamine against (f) *S. aureus* ATCC 29737 and (g) MRSA DB 21595 strains. For a comparison ES gelatin mats containing 2% LPEI before crosslinking with dopamine was also included in the figure. (h) Antimicrobial durability of the mats to leaching, tested against the *MRSA DB21595* strains. For a comparison results from Aquacel[®] Ag mats was also shown

Supporting Information

Insight into Membrane Selectivity of Linear and Branched Polyethylenimines and Their Potential as Biocides for Advanced Wound Dressings

Stephen John Fox, Mobashar Hussain Urf Turabe Fazil, Chetna Dhand, Mayandi Venkatesh, Eunice Tze Leng Goh, Sriram Harini, Christo Eugene, Rayne Rui Lim, Seeram Ramakrishna, Shyam Sunder Chaurasia, Roger W Beuerman, Chandra Shekar Verma, Navin Kumar Verma, Xian Jun Loh and Rajamani Lakshminarayanan*

Dr. C. Dhand, Mr. M. Venkatesh, Ms. S. Harini, Ms. E. T. L. Goh, Mr. C. Eugene, Prof. R. W. Beuerman, Dr. R. Lakshminarayanan
Anti-Infectives Research Group
Singapore Eye Research Institute, Singapore 168751.
Email: lakshminarayanan.rajamani@seri.com.sg

Ms. Rayne Rui Lim and Dr. Shyam Sunder Chaurasia
Department of Ophthalmology Veterinary Medicine & Surgery,
University of Missouri, Columbia, MO 65212, USA.

Dr. X. J. Loh
Institute of Materials Research & Engineering, A*STAR (Agency for Science, Technology and Research), 2 Fusionopolis Way, Innovis, #08-03, Singapore 138634.
Email: lohxj@imre.a-star.edu.sg

Dr. M. H. U. T. Fazil and Dr. N. K. Verma
Lee Kong Chian School of Medicine, Experimental Medicine Building, Nanyang Technological University, Singapore 636921.
Email: nkverma@ntu.edu.sg

Dr. S. J. Fox and Dr. C. S. Verma
Bioinformatics Institute,
Agency for Science, Technology and Research (A*STAR),
Singapore 138671.

Prof. S. Ramakrishna
Department of Mechanical Engineering
National University of Singapore, Singapore 117576.

Dr. R. Lakshminarayanan and Prof. R. W. Beuerman
SRP in Neuroscience and Behavioral Disorders, Duke-NUS Graduate Medical School,
Singapore 169857.

Experimental Details

Antimicrobial susceptibility testing: MICs of polymers/antibiotics were determined using the microdilution method in 96 well microtitre plates (SPL Life Sciences Co., Ltd, Korea), following the guidelines of Clinical and Laboratory Standards Institute (CLSI).^[1] Antimicrobial activity of gelatin mats or gelatin mats loaded with 2% (w/w) LPEI and crosslinked with polydopamine were evaluated by both Kirby-Bauer disc diffusion method following the protocols reported by Tkachenko and Karas for commercial wound dressings.^[2] For assessing the bacterial/yeast viability, a 2 ml of bacterial suspensions in MH broth (pH~5.8±0.2) or yeasts suspensions in Sabaroud's Dextrose (SD) broth (at 0.5 McFarland standards) were exposed 1 cm² of the gelatin mats for 24 h. 100 µL aliquots of the suspension was diluted serially (10-fold) in 10 mM PBS solution and pour plated on MH or SD agar plates and incubated at 37 °C for 48 h for bacteria and 72 h for yeasts. The colony forming units were enumerated and reported. For Time-kill kinetics, the same protocol was followed as above but the aliquots of broth were withdrawn at specific time intervals (0, 0.5, 1, 2, 4, 8 and 24 h), diluted, poured onto MHA or SDA and incubate at 37 °C for 48/72 h. The mean CFU/ml for each aliquot was enumerated as before.

Membrane Depolarization Assay: Changes in membrane potential of *S. aureus*, *P. aeruginosa* and *C. albicans* upon addition of polymers were monitored by the release of potential sensitive probe diS-C3-5 dye, as reported by previously by our group.^[3] Briefly, 1 ml of overnight grown mid-log phase microbial cells in 5 mM HEPES buffer (pH 7.0) was mixed with 10 µM diS-C3-5 and incubated at 37 °C for 1–2 h in a thermo shaker. 800 µL of the dye-loaded cell suspension was transferred to a stirred quartz cuvette and the change in fluorescence intensity was monitored at an emission wavelength (λ_{em}) of 670 nm (excitation wavelength, 622 nm) using a Quanta Master spectrofluorimeter (Photon Technology International, NJ, USA). The excitation and emission bandwidths were set at 1 and 2 nm, respectively. Once a constant baseline fluorescence level was achieved, 10 µL of a

concentrated polymer solution in HEPES buffer was added so that the final concentration of the polymers range from $0.5 - 16 \times \text{MIC}$. The change in fluorescence intensity after the addition of polymers was monitored continuously for 30 minutes.

Scanning Electron Microscopy: The morphology and diameter of the electrospun fibers were studied by scanning electron microscope (SEM) (Quanta 200F, FEI, Oregon, USA). Prior to imaging with SEM, the mats were sputter coated with gold (JEOL JFC-1200 fine coater, Japan). Imaging was done at an accelerating voltage of 8 kV.

Confocal microscopic analysis of dermal fibroblast cells: For confocal microscopy, human dermal fibroblast cells were grown on nanofibre-coated glass coverslips for 24 h and then fixed in 3% paraformaldehyde. Cells were cultured on coverslips with or without nocodazole as control. After washing with PBS, cells were stained with FITC conjugated α -tubulin (Sigma-Aldrich) and Alexa Fluor 569 phalloidin (Molecular Probes®) to visualize the morphological changes in cytoskeletal systems and Hoechst (Sigma-Aldrich) to visualize the nuclei. Coverslips were mounted on glass slides using Fluoromount™ Aqueous mounting (Sigma-Aldrich). Confocal imaging was carried out by a laser scanning microscope (Zeiss LSM710-Meta, Carl Zeiss Microimaging Inc., NY, USA) using a $\times 40$ oil immersion objective lens. Excitation wavelengths used were 405 nm, 490 nm and 561 nm and emission filters were BP 465 nm, BP 520 nm and 572-754 nm respectively. At least 20 different microscopic fields were analyzed for each sample.

MTS-based cell viability assay: Cell viability was determined using CellTier 96® Aqueous One solution cell proliferation assay kit according to the manufacturer's instruction (Promega Corporation, Madison, WI). This assay evaluates mitochondrial function by measuring ability of viable cells to reduce MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)) into a quantifiable blue, insoluble formazan product. Briefly, human dermal fibroblast cells were seeded in 96-well

plates (2×10^3 cells/well) and incubated for 24 h at 37°C and 5% CO₂. Cells were then treated with various concentrations of polymers (62.5 µg/ml – 2 mg/ml. For the determination of cytotoxicity of polymers-loaded electrospun mats, cells were incubated with fibre mats-coated coverslips placed in a 12-well plate containing 500 µl of cell culture medium. At the end of the treatment period (after 24 h), 20 or 50 µl of MTS tetrazolium solution was added into 96-well or 12-well plates and further incubated for 2 h at 37 °C. Subsequently, the absorbance was measured at 490 nm using a microplate reader (Infinite M200 Pro, Tecan, Switzerland) and the relative cell viability was calculated. Each treatment was performed in triplicate and reported.

MD Simulations: Molecular dynamics uses a potential energy function optimised from empirical measurements to describe a collection of atoms described as balls with a certain size, mass and charge, connected by springs, in the following way,

$$E_{\text{tot}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{dihedral}} + E_{\text{vdw}} + E_{\text{electrostatics}} .$$

The first three terms are the bonding energy terms, where the bonds and angles are described by a harmonic potential and the diheadrals by a cosign funtion. The last two are the non-bonding energy terms, and are described by a 6-12 Lennard-Jones potential for the VDW's and a coulomb potential for the electrostatics. With the potential energy for a system of atoms computed using a potential similar to that above, and integrating over Newtons Law of motion, $F = M \cdot A$ (using a thermostat to regulate the temperature), the thermodynamic propogation of that system can be modelled.

All simulations were carried out in the Amber14 package, using the lipid14 and GAFF force fields. Parameters for the PEI polymer subunits were generated using GAFF atom types and RESP partial charges. A single polymer unit was 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, using NWChem. The BPEI monomers were constructed from a combination of smaller units, namely $\text{CH}_2\text{-CH}_2\text{-NH}_3^+$ (R-NH_2), $\text{R-NH}_2^+\text{-R}$ (R-NH-R) and NHR_3^+ (NR_3) units. Amber library files were then created for the monomers, allowing polymers of any length to be built (in this study, 20 LPEI monomers and 3 BPEI monomers (corresponding to backbone length of 19 monomer LPEI units). The alternate charged/neutral polymers were built from protonated and unprotonated units. The two membrane models used were of a POPC/Cholesterol model in a ratio of 10:8, and a POPE/POPG model in a ratio of 4:1, with a total of 198 and 190 lipids making up the bilayer respectively. The area per lipid for the POPC/Chol bilayer is $41.4 \text{ \AA}^2$, with a thickness of $41.3 \text{ \AA}$ and box dimensions of $66.5 \text{ \AA}$ by $63.6 \text{ \AA}$ by $145.1 \text{ \AA}$. The addition of cholesterol is well known for reducing the area per lipid of the pure POPC membrane ($\sim 64 \text{ \AA}^2$). The area per lipid of the POPE/POPG membrane is $54.9 \text{ \AA}^2$ with a thickness of $43.5 \text{ \AA}$ and box dimensions of $79.8 \text{ \AA}$ by $63.8 \text{ \AA}$ by $119.3 \text{ \AA}$.

Before running production simulations, the membranes were equilibrated so that energies, densities and structures were stable. The equilibration of the system involved relaxation of the solvent, heating of the system from 0-310 K over 105 ps in the NVT ensemble, followed by a 100 ns simulation in the NPT ensemble at 310 K. Eight systems were set up, four in the mammalian membrane model and four in the bacterial membrane model. The polymers were inserted into the membrane perpendicular to the plane of the bilayer, so that the membrane was complete bisected by the polymers, with a lipid-polymer ratio of 15:1 for LPEI and 22:1 for BPEI due to its larger size. The systems charge was neutralized with counter ions (Cl^-) and were then slowly minimised to reduce steric clashes from the insertion, and re-equilibrated using the above procedure. Production simulations were ran for 300 ns at 310 K in the NPT ensemble. The end point of the production simulations for each polymer in the two membranes are represented in **Figure 1** of the manuscript.

Durability of LPEI crosslinked mats: The antimicrobial durability of LPEI crosslinked mats was performed as per the ASTM protocol.^[4] The ES mat was cut into small square pieces of ~10 mg each and dipped in 1 mL of PBS in 24 well nunc dishes with constant shaking. After 1 or 7 days, two mats (duplicate sets) were removed from the nunc dishes, rinsed gently with distilled water for 4-5 times, placed onto 1 ml of bacterial inoculum (MRSA DB21595) for 24 h. 100 μ L aliquots of the suspension was diluted serially (10-fold) in 10 mM PBS solution and pour plated on MH plates and incubated at 37 °C for 48 h and number of viable colonies was enumerated.

References

- [1]. Clinical and Laboratory Standard Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Information Supplement*, CLSI document M100-S18, Wayne, PA, Clinical and Laboratory Standard Institute, **2008**.
- [2]. O. Tkachenko, A. Karas, *J. Antimicrob. Chemother.* **2012**, 67, 1697.
- [3]. Y. Bai, S. Liu, J. Li, R. Lakshminarayanan, P. Sarawathi, C. Tang, D. Ho, C. Verma, R. W. Beuerman, K. Pervushin. *J. Biol. Chem.* **2012**, 287, 26606.
- [4]. Standard test methods for determining antimicrobial activity of antimicrobial agents under dynamic conditions. E2149-13A.

Figure S1

Figure S1. Mode of interactions between linear (A) and branched (B) PEIs with the zwitterionic POPC. Note that the backbone of LPEI is shielded away from the cationic tertiary ammonium group present in the lipid, thus forming a weak polymer-lipid complex. The average distance between LPEI backbone nitrogen and phosphate head group was 3.6 Å. However, in BPEI, the side chain amino group readily forms complex with the phosphate head group and the backbone was not constrained by the repulsive interactions with the tertiary ammonium group. The average distance between BPEI side chain nitrogen and phosphate head group was 3.1 Å.

Figure S2

Figure 2. Penetration of water molecules across POPC/Cho (a) and POPE/PG (b) bilayers after interaction of Alt_LPEI. Water molecules are shown by space filling model.

Figure S3

Figure S3. Concentration-dependent cytoplasmic membrane depolarization of *P. aeruginosa* ATCC 9027 cells by (a) LPEI and (b) BPEI. The black arrows indicate the time of addition of polymers.

Figure S4

Figure S4. Polymers-induced cytoplasmic membrane depolarization of *S. aureus* DM4001R cells by (a) LPEI and (b) BPEI. The black arrows indicate the time of addition of polymers. (c) Assessment of bacterial viability in the presence of polymers (at 2× MIC) after 30 min. The results indicate that the loss of membrane potential is associated with the bacterial lethality by the polymers.

Figure S5

Figure S5. Concentration-dependent cytoplasmic membrane depolarization of *C. albicans* ATCC 2091 cells by (a) LPEI and (b) BPEI. The black arrows indicate the time of addition of polymers.

Figure S6

Figure S6. Dynamic monitoring of cell cytotoxicity by xCELLigence. (a) Changes in cell impedance upon addition of PBS, Triton-X100 and aminoglycoside antibiotics. The black arrow indicates the time of addition of the controls. The concentration-dependent changes in normalized CI values for LPEI (b) and BPEI (c) in comparison with PBS is represented as bar graphs. * $P < 0.05$ and **** $P < 0.0001$ after Dunnett's test for multiple comparisons.

Figure S7

Figure S7. ATR-FTIR spectra of ES gelatin mats containing PEIs crosslinked with dopamine. (a) pristine ES gelatin, (b) LPEI-loaded ES gelatin crosslinked with polydopamine and (c) BPEI-loaded ES gelatin crosslinked with polydopamine.

Figure S8

Figure S8. Antimicrobial activity of polydopamine crosslinked electrospun gelatin mats containing 2% LPEI (ES_Gel_2%LPEI) against Gram-positive and yeasts strains. For a comparison the disc diffusion assay for pristine gelatin mats (ES_Gel) is also shown.

Table S1 Minimum inhibitory concentration of linear and branched PEIs against a panel of Gram-negative pathogens. The source of clinical isolates is indicated in parentheses. The antimicrobial activity of the polymers against tobramycin-resistant strains is highlighted in bold.

Gram-negative bacteria	Minimum Inhibitory Concentration in μM ($\mu\text{g/mL}$)		
	BPEI	LPEI	Tobramycin
<i>Acinetobacter baumannii</i> ATCC 19606	>13 (>128)	>6.4 (>128)	4.4 (2)
<i>Citrobacter braakii</i> ATCC 6750	>13 (>128)	>6.4 (>128)	2.2 (1)
<i>Escherichia coli</i> ATCC 10536	>13 (>128)	>6.4 (>128)	2.2 (1)
<i>Escherichia coli</i> ATCC 8739	>13 (>128)	>6.4 (>128)	4.4 (2)
<i>Escherichia coli</i> ATCC 25922	>13 (>128)	>6.4 (>128)	4.4 (2)
<i>Klebsiella pneumonia</i> ATCC 10031	>13 (>128)	>6.4 (>128)	< 1.1 (<0.5)
<i>Klebsiella pneumonia</i> ATCC 27736	>13 (>128)	>6.4 (>128)	< 1.1 (<0.5)
<i>Klebsiella pneumonia</i> ATCC 4352	>13 (>128)	>6.4 (>128)	< 1.1 (<0.5)
<i>Klebsiella pneumoniae</i> DM4299 (eye)	>13 (>128)	>6.4 (>128)	< 1.1 (<0.5)
<i>Pseudomonas aeruginosa</i> ATCC 15442	6.4 (64)	>6.4 (>128)	< 1.1 (<0.5)
<i>Pseudomonas aeruginosa</i> ATCC 27853	6.4 (64)	3.2 (64)	< 1.1 (<0.5)
<i>Pseudomonas aeruginosa</i> ATCC 9027	6.4 (64)	3.2 (64)	< 1.1 (<0.5)
<i>Acinetobacter baumannii</i> DR 43357 (sputum)	>13 (>128)	>6.4 (128)	2.2 (1)
<i>Citrobacter koseri</i> DM0004432R	>13 (>128)	>6.4 (128)	>34.2 (>16)
<i>Escherichia coli</i> DB0016027R (blood)	>13 (>128)	>6.4 (128)	>34.2 (>16)
<i>Pseudomonas aeruginosa</i> 07DM023376 (eye)	3.2 (32)	3.2 (64)	< 1.1 (0.5)
<i>Pseudomonas aeruginosa</i> PAE230DR4877/07 (sputum)	3.2 (32)	3.2 (64)	>34.2 (>16)
<i>Pseudomonas aeruginosa</i> 412DR18531 (unknown)	1.6 (16)	1.6 (32)	>34.2 (>16)
<i>Pseudomonas aeruginosa</i> DM023257 (eye)	6.4 (64)	3.2 (64)	< 1.1 (0.5)
<i>Pseudomonas aeruginosa</i> DU14476/07 (urine)	3.2 (32)	3.2 (64)	>34.2 (>16)
<i>Pseudomonas aeruginosa</i> DM023155 (eye)	6.4 (64)	3.2 (64)	< 1.1 (0.5)
<i>Pseudomonas aeruginosa</i> DR5790 (wound)	3.2 (32)	3.2 (64)	>34.2 (>16)
<i>Pseudomonas aeruginosa</i> DM0004150R (PsD) (eye)	6.4 (64)	3.2 (64)	< 1.1 (0.5)

Table S2. Minimum inhibitory concentration of linear and branched PEIs against a panel of Gram-positive pathogens. The source of clinical isolates is indicated in parentheses. The antimicrobial activity of the polymers against tobramycin-resistant strains is highlighted in bold.

Gram-positive bacteria	Minimum Inhibitory Concentration in μM ($\mu\text{g/ml}$)		
	BPEI	LPEI	Tobramycin
<i>Bacillus cereus</i> ATCC 11778	1.6 (16)	1.6 (32)	4.4 (2)
<i>Bacillus subtilis</i> ATCC 6633	6.4 (64)	3.2 (64)	< 1.1 (<0.5)
<i>Enterobacter aerogenes</i> ATCC 13048	>13 (>128)	>6.4 (>128)	2.2 (1)
<i>Enterococcus faecalis</i> ATCC 29212	6.4 (64)	>6.4 (>128)	>34.2 (>16)
<i>Enterococcus faecalis</i> ATCC 700802	12.8 (128)	>6.4 (>128)	>34.2 (>16)
<i>Enterococcus hirae</i> ATCC 9790	3.2 (32)	>6.4 (>128)	>34.2 (>16)
MRSA ATCC 43300	1.6 (16)	1.6 (32)	> 34.2 (16)
MRSA ATCC 700699	1.6 (16)	3.2 (64)	> 34.2 (16)
MRSA ATCC BAA-38	>13 (>128)	6.4 (128)	< 1.1 (<0.5)
<i>Staphylococcus aureus</i> ATCC 29213	1.6 (16)	1.6 (32)	2.2 (1)
<i>Staphylococcus aureus</i> ATCC 6538	1.6 (16)	1.6 (32)	2.2 (1)
<i>Staphylococcus aureus</i> ATCC 29737	1.6 (16)	1.6 (32)	2.2 (1)
<i>Staphylococcus epidermidis</i> ATCC 12228	0.4 (4)	1.6 (32)	1.1 (0.5)
MRSA DR42412 (sputum)	1.6 (16)	1.6 (32)	>34.2(>16)
MRSA DB21455 (eye)	1.6 (16)	1.6 (32)	>34.2(>16)
MRSA DB09808R (eye)	1.6 (16)	1.6 (32)	> 34.2(>16)
MRSA DB57964/04 (blood)	1.6 (16)	1.6 (32)	>34.2(>16)
MRSA DB68004 (blood)	1.6 (16)	1.6 (32)	> 34.2(>16)
MRSA DB21595 (wound)	1.6 (16)	1.6 (32)	>34.2(>16)
MRSA DB6506 (blood)	1.6 (16)	1.6 (32)	2.2 (1)
<i>Staphylococcus aureus</i> DM0004001R (eye)	1.6 (16)	0.8 (16)	2.2 (1)
<i>Staphylococcus aureus</i> DM4299 (eye)	1.6 (16)	0.8 (16)	<1.1 (<0.5)
<i>Staphylococcus aureus</i> DM0004583R (eye)	1.6(16)	0.8 (16)	2.2 (1)
<i>Staphylococcus aureus</i> DM0004400R (cornea)	1.6 (16)	1.6 (32)	<1.1 (<0.5)

Table S3. Minimum inhibitory concentration of linear and branched PEIs against Yeasts and filamentous fungus.

S.No:.	Fungal/Yeasts strains	Minimum Inhibitory Concentration in μM ($\mu\text{g/ml}$)	
		BPEI	LPEI
1	<i>Candida albicans</i> ATCC 2091	1.6 (16)	0.8 (16)
2	<i>Candida abicans</i> ATCC 10231	1.6 (16)	0.8 (16)
3	<i>Candida albicans</i> ATCC 24433	1.6 (16)	3.2 (64)
4	<i>Candida albicans</i> DF0001976R (colon)	1.6 (16)	1.6 (32)
5	<i>Candida albicans</i> DF0002672R (urine)	1.6 (16)	0.8 (16)
6	<i>Aspergillus brasiliensis</i> ATCC 16404	>13 (>128)	>6.4 (>128)

Table S4. Average distance between the charges in zwitterionic lipid head groups (P-N distances) and average N-N distances in the full and partially charged LPEI polymer.

Lipid/polymer		Distance (Å)
POPC	$\text{PO}_4^- \cdot (\text{CH}_2)_2\text{N}(\text{CH}_3)_3^+$	4.6
POPE	$\text{PO}_4^- \cdot (\text{CH}_2)_2\text{NH}_3^+$	4.4
LPEI	$-\text{NH}_2^+ \cdot (\text{CH}_2)_2\text{NH}_2^-$	3.9
LPEI	$-\text{NH}_2^+ \cdot (\text{CH}_2)_2\text{NH}_2^+ \cdot (\text{CH}_2)_2\text{NH}_2^-$	6.8
Alt-LPEI	$-\text{NH}_2^+ \cdot (\text{CH}_2)_2\text{NH}-$	3.1
Alt-LPEI	$-\text{NH}_2^+ \cdot (\text{CH}_2)_2\text{NH} \cdot (\text{CH}_2)_2\text{NH}_2^+$	6.1

Table S5. Number of water molecules that inter the lipid bilayer after interaction with the polymers,. The total number of molecules is estimated by averaging over the last 50 ns of the simulation.

	POPC/Chol	POPE/PG
Membrane	269	237
LPEI	168	430
BPEI	3212	1354
Alt-LPEI	828	1238
Alt-BPEI	3060	1489