SIMPLIFY HEALTHCARE DECISION MAKING BY SIMPLIFYING WOUND CARE OPTIONS

Which Wound Care Factor is Important for Healing?
(select all that apply)

Hemostasis
- □ Platelet aggregation
- □ Degranulation
- □ Fibrin formation

Inflammation
- □ Bacterial burden management
  - □ *Staphylococcus aureus*
  - □ *Pseudomonas aeruginosa*
  - □ Other ESKAPE pathogens
  - □ Antibiotic resistance
  - □ Biofilms
- □ PAMPs/DAMPs
- □ Pain/Irritation/Itching
- □ Lymphocyte infiltration
- □ Neutrophil infiltration
- □ Monocyte infiltration
- □ Differentiation of macrophages

Proliferation
- □ Bacterial burden
- □ Extracellular matrix formation
- □ Pain/Irritation/Itching
- □ Re-epithelialization
- □ Angiogenesis
- □ Desiccation

Tissue Remodeling
- □ Pain/Irritation/Itching
- □ Extracellular matrix remodeling
- □ Vascular maturation

Would you prefer a Single Product that can target numerous wound care factors?
or
Many different products for each of the different wound care factors?

VS.

Value Proposition for product development

BPEI offers a low-cost, off-the-shelf technology that can be easily incorporated into wound lavage solutions, gels, creams, and foams, reducing the cost and complexity of manufacturing wound care products.

Value Proposition for healthcare providers

BPEI provides broad-spectrum properties that can target multiple factors that inhibit healing, simplifying wound care treatment while also improving patient outcomes.

Competitive Advantage.

BPEI replaces multiple products with a single active ingredient. To the best of our knowledge, there are no existing devices, pharmaceuticals, or OTC products that target bacterial pathogens, biofilms, and inflammatory toxins in one product.

Novelty of the Science

Cationic BPEI binds to anionic targets found within pathogens, biofilms, and toxins. Since BPEI is not a peptide or an enzyme, it remains stable in the wound environment. BPEI also functions as a buffer to modulate pH within the wound environment, accelerating wound healing.

Biomaterials for Controlled-Release of Agents with Antimicrobial Properties

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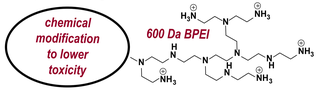
NIH National Institute of Allergy and Infectious Diseases
CDMRP Congressionally Directed Medical Research Programs, Department of Defense

R03AI-142420 R03AI-166402 PR210140

INTRODUCTION

The critical problem to be addressed is that current wound treatments do not have extended activity to prevent bacterial colonization, biofilm formation, and sepsis. Accordingly, the FDA identified non-healing wounds as an area of priority "Due to high unmet need with relatively limited research and funding". Thus, we seek to deliver PEGylated branched polyethylenimines (PEG-BPEIs) to wound infections using biocompatible materials.

Wound treatments must address the underlying factors that inhibit healing, such as (1) drug-resistant pathogens, (2) biofilms, and (3) inflammation from pathogen associated microbial pattern molecules (PAMPs). Data show that one molecule – 600 Da BPEI that is PEGylated to improve safety – is designed to meet these goals. Cationic PEG-BPEI uses electrostatics for binding with anionic sites on Gram-positive and Gram-negative bacteria. PEG-BPEI, and the bioactive moiety 600 Da BPEI, have broad-spectrum activity to counteract (1) antimicrobial resistance (AMR) caused by the Gram-negative LPS layer; (2) AMR caused by Gram-positive cell wall and teichoic acids (3) AMR caused by metallo-β-lactamases; (4) Release of pro-inflammatory cytokines in response to the Gram-negative pathogen associated molecular pattern molecules (PAMPs) LPS and peptidoglycan; (5) Release of pro-inflammatory cytokines in response to the Gram-positive PAMPs teichoic acids and peptidoglycan; and (6) Biofilms formed by Gram-negative pathogens; and (7) Biofilms formed by Gram-positive pathogens.

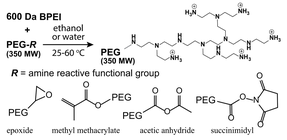


PEGylated 600 Da BPEI (PEG-BPEI)

DIFFERENTIATION FACTORS

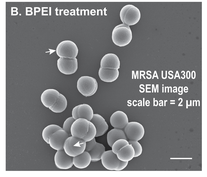
BPEI addresses (1) antimicrobial resistance (AMR) and multi-drug resistance (MDR); (2) biofilms; and (3) pathogen-associated molecular pattern molecules (PAMPs).

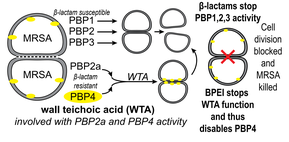
- BPEI is NOT a peptide; thus, it resists proteolysis
- Broad-spectrum activity against:
  - MDR Gram-positive and Gram-negative pathogens
  - Biofilms from Gram-positive and Gram-negative pathogens
  - PAMPs from Gram-positive and Gram-negative pathogens
- Easy and straightforward one-step synthesis
- Hydrophilic properties and very-high water solubility to enable different means of drug delivery
- There are minimal protein binding effects
- Minimal *in vitro* cytotoxicity
- *In vivo* toxicity mitigated with chemical design



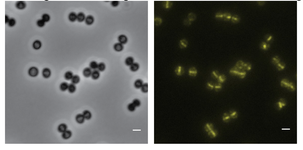
KILLING MRSA

PEG-BPEI, and the bioactive moiety 600 Da BPEI, are antibiotic potentiators, generating a 4- to 256-fold reduction in the minimum inhibitory concentration of β-lactams, cephalosporins, carbapenems, erythromycin, linezolid, and chloramphenicol. Laser-scanning confocal microscopy images show that BPEI binds to the cell-division septum of MRSA. Here, BPEI prevents localization of PBP4 enzymes that are important AMR factors.

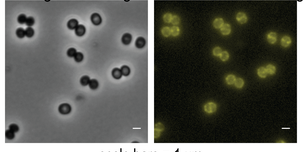




MRSA COL PBP4:YFP without BPEI
A. Brightfield Image B. Fluorescence Image



MRSA COL PBP4:YFP with 64 µg/mL BPEI
C. Brightfield Image D. Fluorescence Image



scale bars = 1 µm

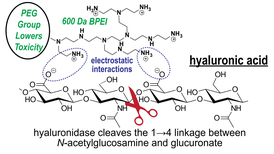
PROPERTIES IDEAL FOR WOUND HEALING

BPEI can simplify wound care. It targets several major causes of poor wound healing, such as the presence of bacteria that aggravate chronic wounds through the action of antibiotic resistance, endotoxins, protease activity, biofilms, and inflammation. Neutralizing these factors allows for tissue regeneration processes leading to the restoration of nerve function and blood vessel networks. Thus, BPEI is a universal agent that could lead to better wound care clinical strategies by reducing the need to tailor patient care based on the wound type and clinical characteristics. Other wound management strategies that center on repair of the skin and underlying tissues would be improved by addressing infection, biofilms, and excessive inflammation with BPEI, properties ideally suited for wound healing.

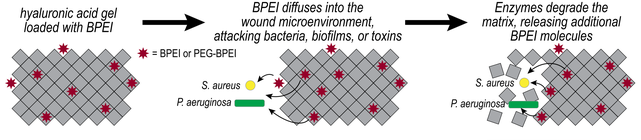
BPEI Property	Target(s)	Mechanism(s) of Action
A. Kills Gram positive bacteria	anionic LTA and WTA	prevents septum formation and cell division
B. Kills Gram negative bacteria	anionic LPS in the OM	disrupts OM integrity, prevents cell division
C. Disrupts AMR mechanisms	anionic LTA, WTA, LPS	blocks PBP2a localization; increases drug influx
D. Disables metallo-β-lactamases	metalloenzymes	chelates essential zinc cations
E. Disrupts bacterial biofilms	anionic EPS, LTA, eDNA	binds to and disperses the biomass
F. Neutralizes Gram(+) PAMPs	anionic LTA; whole cells	hinders TLR2 signaling and cytokine release
G. Neutralizes Gram(-) PAMPs	anionic LPS	hinders TLR4 signaling and cytokine release

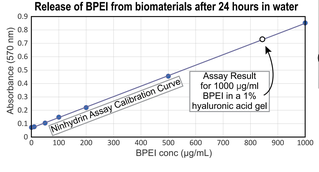
HYALURONIC ACID HYDROGELS LOADED WITH BPEI

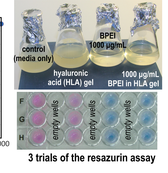
BPEI could adress the medical need – developing new products for wound healing – through the use of non-immunogenic hyaluronic acid biomaterials. As a key component of the extracellular matrix, hyaluronic acid can encapsulate and deliver therapeutic agents to wounds. As wound dressing materials, the strong hydrophilicity of hyaluronic acid keeps tissues hydrated during the healing process and supports the growth of new regenerative tissue. Chemical crosslinking of hyaluronic acid creates biodegradable scaffolds for wound repair that can be combined with antibiotics to kill pathogens. In this project, sponges and gels will not contain any chemicals other than collagen, hyaluronic acid, and BPEI. Crosslinking functions are performed by BPEI itself.



hyaluronidase cleaves the 1→4 linkage between *N*-acetylglucosamine and glucuronate







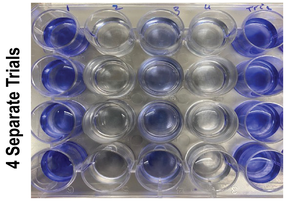
The presence of BPEI in solution can be quantified with the ninhydrin assay, which is insensitive to the presence of hyaluronic acid, show that 83% of BPEI is recovered from hyaluronic acid gels within 24 hours. MRSA USA 300 killing was evaluated using a resazurin cellular metabolism assay. Resazurin (blue in color) is reduced by NADH to resorufin (pink in color). The persistence of the blue color indicates growth inhibition and this was observed in MRSA USA 300 cells exposed to 1000 µg/mL BPEI delivered by hyaluronic acid gel .

BIOFILM DISRUPTION ASSAY

Extracellular polymeric substance (EPS) of MRSA is composed of extracellular DNA and teichoic acids. BPEI binds to these species and weakens biofilm architecture, allowing biofilm dispersal.

1	2	3	4	Ingredients
X	X			Ethanol, 100 g/L
X	X	X	X	Acetic Acid, 59 g/L
X	X	X	X	Sodium acetate, 30 g/L
X		X		Benzalkonium chloride, 1.3 g/L
	X		X	600 Da BPEI, 1.3 g/L

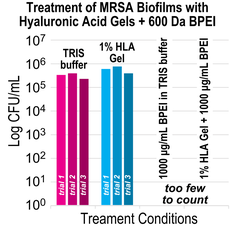
Solution: 1 2 3 4 buffer



MRSA biofilms were treated and washed. The remaining biomass was stained with crystal violet and dissolved with 30% acetic acid. The lack of blue color from solutions 2 and 4 show that BPEI washed away the biofilm and thus is a superior antibiofilm agent.

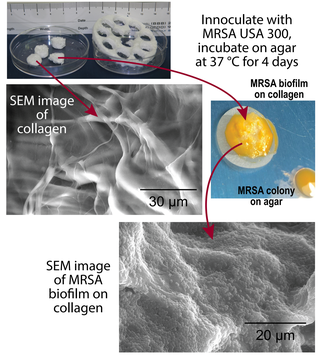
KILLING BACTERIAL BIOFILMS

The susceptibility of MRSA USA 300 biofilms to BPEI was evaluated using serial dilution and enumerating the number of viable bacterial colonies on agar plates.Data collected by our team demonstrate that hyaluronic acid gels loaded with BPEI attack and kill MRSA biofilms with a 5-log reduction in MRSA CFUs.



MRSA BIOFILM SCAFFOLD

Biofilms were grown on collagen sponges. The images and data below show the MRSA biofilm within the collagen fiber network.



IN VIVO DATA

PEGylating one of the primary amines on 600 Da BPEI lowers single-dose acute toxicity. Toxicity data were collected by TransPharm Preclinical Solutions Inc., a contract research lab. The acute toxicity LD_0, or maximum tolerable dose (MTD), was evaluated over 3 days using female ICR mice with daily subcutaneous q24h dosing. For 600 Da BPEI, the MTD is 25 mg/kg. A single 350 MW PEG on BPEI (PEG350-BPEI) increased MTD to 75 mg/kg. Thus, sub-cutaneous PEG-BPEI, for instance applied to a wound or burn with exposed tissue layers, has lower acute toxicity and is safer to use than 600 Da BPEI.

acute toxicity > 75 mg/kg

Average Wound Area (*n* = 4, normalized to day 1)

Day	MRSA + saline	MRSA + 50 µg PEG350-BPEI	MRSA + 250 µg PEG350-BPEI	MRSA + 1250 µg PEG350-BPEI
1	**1.00**	**1.00**	**1.00**	**1.00**
2	**0.93**	**0.92**	**0.87**	**0.79**
4	**0.86**	**0.83**	**0.72**	**0.70**

REFERENCES

Google Scholar URL for Rice Lab publications

OFFICE OF TECHNOLOGY COMMERICIALIZATION
The UNIVERSITY *of* OKLAHOMA

ANTIBIOTIC AND ANTI-INFLAMMATORY COMPOSITIONS AND METHODS OF USE
Tech ID: 2018-013 | PCT/US21/70485

METHODS OF TREATING BACTERIAL INFECTIONS WITH PENAM β-LACTAM ANTIBIOTICS AND BRANCHED POLY(ETHYLENIMINE) US patent US10953040B2

This work is possible due to contributions from University of Oklahoma Prof. Helen Zgurskaya, Prof. Carolyn Ibberson, Prof. Zhibo Yang, Prof. Daniel Glatzhofer, and Oklahoma State University Prof. Karen Wozniak. We want to thank Dr. Phil Bourne and acknowledge the use of the Protein Production Core (PPC) at the University of Oklahoma.