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Amino Acid Block Copolymers with Broad Antimicrobial Activity and Barrier Properties[a]

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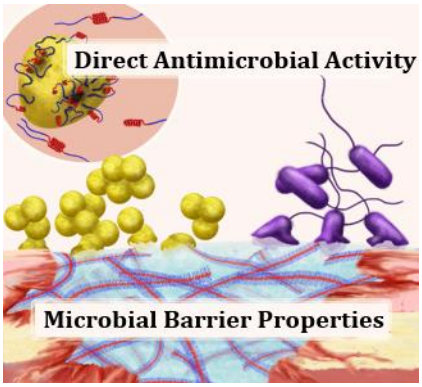
[a] **Supporting Information** is available online from the Wiley Online Library or from the author.

Antimicrobial properties of a long-chain, synthetic, cationic and hydrophobic amino acid block copolymer are reported. In 5 and 60 minute time-kill assays, solutions of $K_{100}L_{40}$ block copolymers (poly(L-lysine·hydrochloride)$_{100}$-*b*-poly(L-leucine)$_{40}$) at concentrations of 10 to 100 μg/mL show multi-log reductions in colony forming units of Gram-positive and Gram-negative bacteria, as well as yeast, including multidrug-resistant strains. Driven by association of hydrophobic segments, $K_{100}L_{40}$ copolymers form viscous solutions and self-supporting hydrogels in water at concentrations of 1 wt% and 2 wt%, respectively. These $K_{100}L_{40}$ preparations provide an effective barrier to microbial contamination of wounds, as measured by multi-log decreases of tissue-associated bacteria with deliberate inoculation of porcine skin explants, porcine open wounds, and rodent closed wounds with foreign body. Based on these findings, amino acid copolymers with the features of $K_{100}L_{40}$ can combine potent, direct antimicrobial activity and barrier properties in one biopolymer for a new approach to prevention of wound infections.



Direct antimicrobial activity and microbial barrier properties provided by a single, long-chain lysine-leucine block copolymer. These amino acid block copolymers are active against Gram-positive and Gram-negative bacteria, as well as yeast in vitro and form viscous solutions and hydrogels that can block microbial contamination of wounds in vivo.

1. Introduction

Human skin is a specialized organ serving to divide outside from inside.[1–4] One critical function is the protection of underlying tissues from microbial invasion. Skin performs this work by a combination of direct antimicrobial activities and microbial barrier properties. Disruption of our natural defenses by surgery, trauma, or disease, exposes deeper tissues to an array of microbial pathogens. This can set off a chain of events culminating in focal infection, and possible life-threatening sepsis.[1,5–24] Until wound closure, prevention relies on temporary replacement of antimicrobial activities and barrier properties. In current standard of care, the former is typically provided by systemic or local antimicrobials (antibiotics and antiseptics) and the latter by wound dressings and bandages. Incorporation of antimicrobial agents, such as silver, into hydrogels or other dressings delivers both functions in a single composition.[25,26] Here, we report that both direct antimicrobial activities and microbial barrier properties can be designed into a single multifunctional biopolymer.

Cationic antimicrobials, both natural and man-made, have been widely studied and are active ingredients in a variety of healthcare products.[27–48] While diverse in structure, cationic antimicrobials are unified by the display of one or more positive charges at physiologic pH that promote binding to multiple negatively charged structures on microbial surfaces. They include antiseptics, such as quaternary ammonium compounds;[28–31] biguanides (e.g. chlorhexidine and polihexanide);[32–35] a few antibiotics (e.g., polymyxins);[36–40] and most naturally occurring antimicrobial peptides (AMPs).[41–48] Cationic antimicrobials are multimodal in mechanism(s) of action, binding multiple molecular targets and disrupting multiple cellular functions. They are sometimes called “membrane active agents” because they operate, at least in part, by disrupting

the function and/or structure of microbial membranes. Typically, cationic antimicrobials display broad-spectrum effectiveness against both Gram-positive and Gram-negative bacteria.

A variety of synthetic polymers have been explored as antimicrobials, and reviews of advances have been published.[49–52] For use of such polymers in medical settings, degradability in vivo is important. Recent progress has been made. For example, Hedrick and Yang have developed degradable antimicrobial micelles and hydrogels based on block copolymers containing carbonate linkages.[51,53] Synthetic cationic polypeptides, especially those containing an abundance of L-lysine residues, can display antimicrobial activity and are attractive for their degradability.[54,55] Recently, this work has been expanded to include soluble statistical copolymers of lysine with various hydrophobic amino acids,[56] graft copolymers on other polymer or particle substrates,[57–59] and chemically cross-linked hydrogels.[60,61] These studies point to the potential of using advanced synthetic biopolymers to combine antimicrobial activity with desirable chemical properties such as degradability. As the field progresses, additional studies will be required to better understand and optimize performance of antimicrobial biopolymers in vivo.

In a complementary area of research, Deming and colleagues have extensively studied long-chain, diblock amino acid copolymers (copolypeptides) containing discrete charged and hydrophobic segments. These diblock amino acid copolymers can be designed to form hydrogels with tunable physical properties, including stiffness and porosity. They also possess other characteristics that may be beneficial for healthcare applications,[62–69] including degradability in vivo,[69] deformability and injectability,[62] and gel formation without the need for chemical crosslinking.

To address the urgent need for prevention of infections in the era of antibiotic resistance, we sought to develop amino acid block copolymers that would be broadly antimicrobial, and would fill and coat tissues to prevent microbial contamination of wounds. Preliminary studies demonstrated that diblock amino acid copolymers, containing cationic and hydrophobic blocks, could show substantial antimicrobial activity.[70–73] A representative long-chain amino acid block copolymer of sequence poly(L-lysine·hydrochloride)$_{100}$-*b*-poly(L-leucine)$_{40}$ ($K_{100}L_{40}$; **Figure 1**) was selected for larger scale synthesis and more detailed evaluation of properties, both in vitro and in vivo. Here, we report that $K_{100}L_{40}$ copolymers demonstrate direct antimicrobial activity against a broad array of potential pathogens in vitro, and that these same copolymers, at higher concentrations, make viscous solutions and hydrogels that protect wounds from microbial contamination in vivo. With this combination of advantageous properties, cationic and hydrophobic amino acid block copolymers, including $K_{100}L_{40}$, may offer a new approach for the prevention of infections in a variety of settings, including the wounds of surgery, trauma, and chronic disease.

2. Experimental Section

2.1. Synthesis and chemical analysis of $K_{100}L_{40}$ amino acid block copolymers.

$K_{100}L_{40}$ block copolymers were synthesized by contract manufacturer, Bachem, Inc. (Vista, CA). The block copolymerization was accomplished by stepwise addition of monomers to benzyl amine initiator under high vacuum at a low temperature.[68] N_{ε}-trifluoroacetyl-L-lysine N-carboxyanhydride (TFA-K NCA) (Isochem) was dissolved in a 1:1 (v:v) mixture of anhydrous DMAc and diglyme with an appropriate amount of initiator. Upon completion of the first block, the second monomer, L-leucine N-carboxyanhydride (L NCA) (Isochem), was added in 1:1 (v:v) DMAc and diglyme. Once monomer consumption was complete, the protected diblock copolymers

were precipitated into hexanes, and then dissolved into a minimal amount of THF, and re-precipitated into hexanes three additional times. Protected copolymers were then dried under vacuum. The trifluoroacetyl groups of the amino acid copolymers were deprotected using potassium carbonate at 50 °C in 90% MeOH. Purification was performed by tangential flow filtration with 5 kDa nominal molecular weight cutoff A-screen hydrophilic PES cartridges against pH 2 HCl (aq.), followed by pH 5 HCl (aq.)

Gel permeation chromatography with multi-angle light scattering (GPC-MALS) was performed using differential refractive index (Wyatt Optilab T-rEX), and light scattering detectors (Wyatt miniDAWN TREOS), a mobile phase of hexafluoroisopropanol with 0.1% potassium trifluoroacetate, Jordi Gel X-Stream H_2O column (mixed bed, 500 Å; Jordilabs, Mansfield, MA), and a flow rate of 1.0 mL/min at 45 ^oC. GPC analysis of the initial poly(N_ε-trifluoroacetyl-L-Lysine) chains, $(TFA\text{-}K)_{100}$, showed narrow chain length distributions (typically, $M_w/M_n < 1.1$) and good agreement with target molecular weight. Comparison of chromatograms of $(TFA\text{-}K)_{100}$ and $(TFA\text{-}K)_{100}L_{40}$ showed an effective increase in molecular weight correlating to addition of the poly(L-leucine) segments. A chromatogram of deprotected $K_{100}L_{40}$ is shown in Supporting Information **Figure S1**.

^1H NMR experiments were conducted on a JEOL ECA 500 MHz NMR spectrometer at the University of California, San Diego. The lyophilized samples were dissolved in deuterated trifluoroacetic acid (*d*-TFA). ^1H NMR analysis in *d*-TFA confirmed removal of trifluoroacetyl protecting groups, and the relative amino acid compositions of final copolymers by comparison of integrals of lysine and leucine resonances (Supporting Information **Figure S2**).

2.2. Mechanical analyses of $K_{100}L_{40}$ amino acid block copolymers in aqueous media.

Samples were prepared by direct dissolution of $K_{100}L_{40}$ copolymers in sterile water (Sigma Aldrich W3500) at concentrations up to 30 mg/mL (3 wt%). After initial vortexing, samples were equilibrated at least 24 hours before additional vortexing and centrifugation (4,000 rpm, ~15 min) to remove air bubbles.

Viscosity was assessed using calibrated Ubbelohde viscometers obtained from CANNON-Instruments. The procedure was adapted from ASTM D 446–07. Samples of $K_{100}L_{40}$ were made at 0.5 and 1.0% w/w, and allowed to equilibrate at 37 °C for 30 min before being run. Each measurement was run in triplicate.

Hydrogel formation was assessed visually in a tilt tube assay. Hydrated $K_{100}L_{40}$ copolymer samples at various concentrations were added to 1 dram glass vials (Scientific Specialties B69302) and pulse centrifuged to collect and level material. Samples were inverted and images were taken within 4 seconds post-inversion. Further, sample resistance to probe penetration at a defined depth, or firmness, was assessed by texture analysis using a TA.XT2 Texture Analyzer (Texture Technologies Corp, Scarsdale, NY).[74,75] In a typical experiment, a sample of $K_{100}L_{40}$ (~2 mL) was placed in a 5 mL polypropylene mailing tube (VWR 16465-262) at room temperature. A TA-57 7 mm diameter stainless steel cylinder probe was then lowered into the sample at 1 mm/sec. The depth of the probe was normalized (depth = 0 mm) when the probe registered a positive force, and firmness was defined as the force at a given depth. Measurements were performed in triplicate.

2.3. Antimicrobial studies in vitro and ex vivo.

2.3.1. Microbial time-kill assays in vitro.

Time-kill assays were conducted at R.M. Alden Research Laboratory (Culver City, CA; RMA), based on test methods published by Clinical and Laboratory Standards Institute (CLSI M7-A7, M11-A8) and ASTM International (ASTM E2315-03). Laboratory strains (ATCC) and/or clinical isolates (RMA) were used. RMA isolates were recovered from clinical samples, identified by standard methods, and stored in 20% skim milk at -70 ºC. Before use in studies, microbes were taken from the freezer and transferred at least twice on Trypticase soy agar (TSA) to ensure purity and good growth. Microbial aliquots were added to samples of $K_{100}L_{40}$ copolymers at different concentrations, and incubated for 5 or 60 minutes at room temperature. Samples were then neutralized and plated for Colony Forming Unit (CFU) counts.

2.3.2. Antimicrobial barrier assays on porcine skin ex vivo.

These assays were performed at iFyber (Ithaca, NY) using porcine skin obtained from a USDA approved facility with controlled herds. Porcine skin samples were cut into 10 mm diameter discs, artificially wounded with a 2 mm wide, 1.5 mm deep cavity using a Dremel tool, extensively washed, sterilized using chlorine gas, and washed. Explants were submersed in 1 wt% $K_{100}L_{40}$ in water or in water alone for 15 minutes, and placed into 24 well plates. Explants were then inoculated with 15 to 20 μL of 10^5 CFU of log-phase *P. aeruginosa* (ATCC BAA-47) cultures and incubated at 37 °C. Three hours post-inoculation, tissue explants were "washed" to remove non-adherent bacteria by transferring to 24-well plates containing 2 mL of sterile water per well and placing the plates on a shaker (200 rpm) for 2 min at room temperature. The washing procedure was performed five times. Bacteria were recovered by individually placing explants into tubes containing 2 mL of Dey / Engley broth, vortexing for 10 seconds, and sonicating for 90 seconds.

This recovery procedure was repeated five times, with 60 seconds of rest between repeats. 20 μL of fluid was transferred from each tube and bacteria quantified using standard methods.

2.4. Animal model studies.

All animal model studies were performed by Bridge Preclinical Testing Services (PTS; San Antonio, TX) in a Public Health Service-assured facility. Studies had Bridge PTS Institutional Animal Care and Use Committee (IACUC) and US Army Medical Research and Materiel Command (USAMRMC) Animal Care and Use Review Office (ACURO) approvals and were performed in accordance to *The Guide for the Care and Use of Laboratory Animals*, applicable animal welfare regulations, and other applicable Federal and Department of Defense regulations.

2.4.1. Porcine open wound model.

Yorkshire-cross female pigs (25 to 35 kg) were housed individually and conditioned for 10 days.[76,77] Animals were sedated by intramuscular injection of 0.05 mg/kg atropine and 4.4 mg/kg Telazol followed by intubation and inhalation of 2 to 5% isoflurane mixed with oxygen. The dorsal and lateral thorax were clipped and washed with antibacterial-free soap. Using a trephine, 12 full-thickness wounds, 1 cm in diameter, were evenly created on each side of the thorax (24 total). An epinephrine solution was applied for hemostasis. Wounds were treated with 1.0 mL $K_{100}L_{40}$ (0.5, 1.0, or 2.0 wt% in water) or water and covered in gauze soaked with 1.0 mL $K_{100}L_{40}$ or water. Strips of gauze sufficient to cover 12 wounds (two per pig) were then applied. Wounds were inoculated 15 minutes post-treatment with 10^8 CFU/mL of *S. epidermidis*, *P. aeruginosa* (pig clinical isolate), and *Fusobacterium sp.* (ratio of 1:1:0.5) by saturating the gauze with 60-80 mL of inoculum. *Fusobacterium* are anaerobic bacteria that support the growth of the aerobes, but are not measured in this model. The gauze strips were then covered with an occlusive layer of Saran

Wrap for 15 minutes, then both gauze and Saran Wrap were discarded. A 25 μg/hr fentanyl patch was provided for post-surgical pain management. Tissue biopsies (4 mm punch) were taken near the center of wounds and transferred to a pre-weighed vessel containing neutralizing agent. Biopsy samples were homogenized, serially diluted, and plated for determination of CFU. The following plates were used: TSA for total bacterial counts, Mannitol Salts Agar (MSA) for *S. epidermidis* counts, and *Pseudomonas* Isolation Agar (PIA) for *P. aeruginosa* counts. At the conclusion of the study, animals were sedated (5 mg/kg Telazol) and euthanized (110 mg/kg pentobarbital sodium).

2.4.2. Rodent closed wound model with foreign body.

Sprague-Dawley male rats (300-400 g) were anesthetized and a 2 cm incision made on their back. A blunt probe was used to create a small subcutaneous pocket into which a polypropylene mesh pre-soaked in $K_{100}L_{40}$ (1 wt% in water) or water was inserted. Immediately following insertion of pre-soaked mesh, 1 mL of $K_{100}L_{40}$ or water was applied directly into the wound pocket. Fifteen minutes later, wounds were inoculated with methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 33593) or *P. aeruginosa* (ATCC 27317) and the wound closed using Tissue Glue (3M) and staples. The treatment group consisted of 6 rats and the control group consisted of 8 rats. After 48 hours post-contamination, animals were euthanized and the bacterial burden in the implanted mesh and surrounding tissue was assessed.

3. Results & Discussion

3.1. $K_{100}L_{40}$ solutions demonstrate antimicrobial activity in time-kill assays in vitro.

$K_{100}L_{40}$ amino acid block copolymers were shown to be broadly antimicrobial against Gram-positive and Gram-negative bacteria, as well as yeast. In time-kill assays with 60 minute exposures (**Figure 2**), $K_{100}L_{40}$ at 10 and 100 μg/mL in aqueous solutions caused multi-log reductions in CFU

of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* In a broader survey, $K_{100}L_{40}$ solutions were shown to be active against more than a dozen microbes, including several multidrug-resistant (MDR) strains and clinical isolates (**Table 1**). Notably, at a $K_{100}L_{40}$ concentration of 100 μg/mL, more than 4 log reduction in CFU was observed with *Acinetobacter baumannii*, MDR *A. baumannii*, MDR *P. aeruginosa*, extended spectrum β-lactamase (ESBL) *E. coli*, *Klebsiella pneumoniae*, and *K. pneumoniae* that produce carbapenemase (KPC), all of which can cause clinical challenges.[78] Microbicidal activity was also demonstrated against the yeast *C. albicans* and fluconazole-resistant *C. albicans*. In parallel studies, five minute exposures to 10 and 100 μg/mL of $K_{100}L_{40}$ resulted in 1.8-2.7 log reduction in CFU of *S. aureus* (ATCC 6538; 2 experiments) and 4-6 log reduction in CFU of *P. aeruginosa* (ATCC 27853; 2 experiments).

3.2. $K_{100}L_{40}$ amino acid block copolymers readily form viscous solutions and self-supporting hydrogels.

$K_{100}L_{40}$ copolymers in water demonstrated increased viscosity at concentrations of 0.5 wt% (5 mg/mL) and above and formed self-supporting hydrogels at concentrations of 2 wt% and above. Using an Ubbelohde viscometer, $K_{100}L_{40}$ copolymers at concentrations of 0.5 and 1.0 wt% in water gave average values of 8.5 and 138.4 mm^2/s at 37 °C, respectively; the reference value for water is 0.6959 mm^2/s.[79] As depicted in **Figure 3**, both the 2 wt% and 3 wt% preparations of $K_{100}L_{40}$ demonstrated gel formation, as assessed visually by lack of immediate flow in inverted samples. Quantitative texture analysis on these samples confirmed the trends observed by viscometry and visual assessment of gel formation. These studies indicated increasing resistance to probe penetration from 0.5 wt% through 3.0 wt% (**Figure 3c**). Overall, the formation of viscous solutions and hydrogels with $K_{100}L_{40}$ copolymers was found to be consistent with data on related block copolypeptides from the Deming lab, where physical associations of hydrophobic,

entantiomerically pure poly(L-leucine) segments were found to drive formation of hydrated networks.[53-55]

3.3. $K_{100}L_{40}$ copolymers prepared as viscous solutions and hydrogels prevent microbial contamination of porcine skin explants.

We tested the ability of $K_{100}L_{40}$ copolymer preparations to block microbial contamination of tissues ex vivo in a porcine skin explant model. In this study, $K_{100}L_{40}$ samples prepared as viscous solutions (1 wt% in water) were applied to porcine explants 15 min prior to inoculation with *P. aeruginosa*. Microbial burden was assessed in the skin explants three hours post-inoculation. As shown in **Figure 4**, more than 10^5 CFU *P. aeruginosa* were found on explants pretreated with water, whereas no microbes were detected on explants pretreated with $K_{100}L_{40}$ preparations. The studies suggest that $K_{100}L_{40}$ copolymer preparations can provide a barrier against microbial contamination of tissues.

3.4. $K_{100}L_{40}$ copolymers prepared as viscous solutions and hydrogels prevent microbial contamination of wounds in vivo.

The ability of $K_{100}L_{40}$ copolymer preparations to prevent microbial contamination of wounds was assessed in two distinct animal models. In the first model, porcine open wounds were pretreated with $K_{100}L_{40}$ copolymer preparations and then inoculated with a mixture of *S. epidermidis* and *P. aeruginosa*. As depicted in **Figure 5**, a single application of $K_{100}L_{40}$ (2 wt% in water) fifteen minutes prior to microbial inoculation resulted in a 99.99% (4 log) reduction in CFU of *S. epidermidis* (with no microbes detected) in tissue biopsies at 4 hours post-inoculation (N = 5 wounds/group; $p<0.01$) compared to water controls. Reduction of tissue associated *P. aeruginosa* was also observed with the 2 wt% preparation of $K_{100}L_{40}$ ($p<0.01$).

In the second model, rodent subcutaneous pockets with foreign body (mesh) were pretreated with $K_{100}L_{40}$ copolymer preparations. Fifteen minutes later, wounds were inoculated with methicillin resistant *S. aureus* (MRSA) and closed. After 48 hours, the microbial burdens of wound tissues and mesh were assessed. As depicted in **Figure 6**, pretreatment with $K_{100}L_{40}$ preparations resulted in a 4-5 log reduction in CFU of MRSA versus control in both tissue and mesh. No microbes detected in wounds pretreated with $K_{100}L_{40}$ preparations. In a parallel study, multi-log reductions were also observed in tissue-associated and mesh-associated *P. aeruginosa*.

Effectiveness in these two animal models, one open wound over four hours and one closed wound over 48 hours, suggest that $K_{100}L_{40}$ copolymer preparations can help prevent microbial contamination of wounds in vivo. For potential clinical applications, it may be noteworthy that the formation of hydrogels with $K_{100}L_{40}$ copolymers is driven primarily by association of hydrophobic segments, which can be disrupted by application of shear stress. This property allows these block copolymer preparations to pass readily through small-bore openings of common delivery devices (e.g., syringes, needles, or catheters) and then reform hydrogels.[62,65] Constructed from only two amino acids, these long-chain block copolymers are expected to show a favorable safety profile for local application to tissues. Beyond the scope of this paper, a series of preliminary safety studies in vivo have supported this expectation. Additional safety studies with $K_{100}L_{40}$ and related copolymers are underway. A combination of direct antimicrobial activity, barrier properties, and safety should enable deep tissue applications for the prevention of infections in surgery, trauma, and chronic wounds.

4. Conclusions

The studies presented here are based on the premise that when natural defenses are broken, the optimal approach combines direct antimicrobial activities and barrier properties. $K_{100}L_{40}$ copolymer preparations were evaluated in antimicrobial studies in vitro and in vivo and found to be broadly active against common wound pathogens. They demonstrated direct antimicrobial activity at low concentrations (i.e., 10 and 100 μg/mL in water) against both Gram-positive and Gram-negative bacteria in vitro. Further, the design of $K_{100}L_{40}$ copolymers allowed for preparation and in vivo application of viscous solutions and self-supporting hydrogels (0.5 to 2 wt% in water). At these higher concentrations, $K_{100}L_{40}$ copolymer preparations were found to prevent microbial contamination of wounds. Potent, direct antimicrobial activity and barrier properties present a high hurdle for microbes to overcome.

In this era of antibiotic resistance, new approaches for the prevention of bacterial infections are needed. This is urgent, especially for life-threatening infections that can occur as a result of surgical procedures, trauma, or chronic disease. Today's antibiotics and antiseptics cannot fully address this challenge.[78,80–82] Wounds disrupt skin's natural functions, and may become contaminated with a variety of microbes; it is increasingly likely that these will be multidrug-resistant. Our goal is to replace both direct antimicrobial activities and barrier properties with a biocompatible material until wounds are closed by surgical procedure and/or natural healing processes. We believe that long-chain cationic and hydrophobic block copolypeptides, as exemplified here with $K_{100}L_{40}$, may achieve this goal.

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Keywords: biopolymer, lysine, antimicrobial, antibiotic resistance, hydrogel

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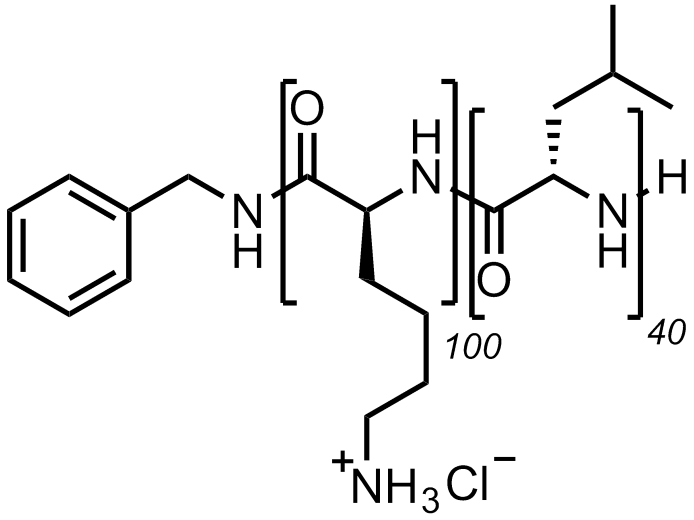

Figure 1. Long-chain amino acid block copolymer of sequence poly(L-lysine·hydrochloride)$_{100}$-*b*-poly(L-leucine)$_{40}$ ($K_{100}L_{40}$).

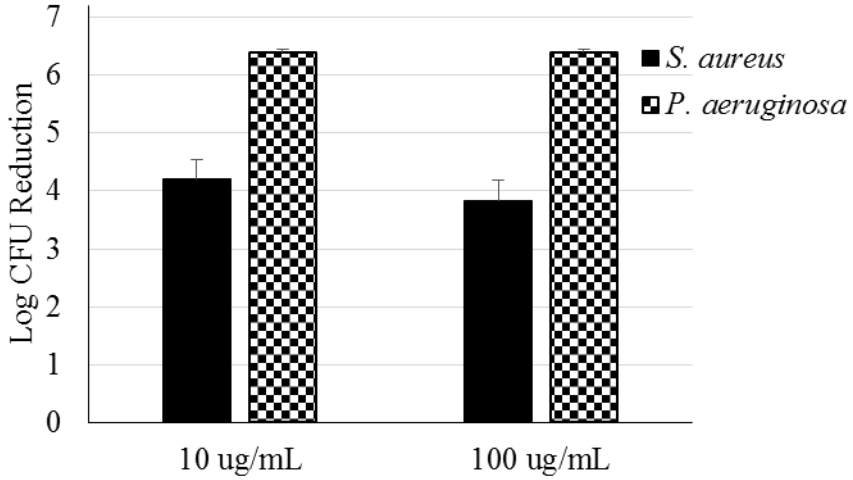

Figure 2. Antimicrobial activity of aqueous $K_{100}L_{40}$ against *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 27853) in vitro. Sixty minute time-kill assays were used to determine microbicidal activity (log CFU reduction) at different sample concentrations. Data were obtained from three separate experiments and are presented as mean + SD.

Table 1. Antimicrobial time-kill assay of $K_{100}L_{40}$ block copolymers against a variety of microbes. A 60 minute time-kill assay was performed with $K_{100}L_{40}$ solutions at 100 μg/mL to determine microbicidal activity (percent reduction of colony forming units, CFU). The term "100%" CFU reduction indicates that no microbes were detected. MDR=multidrug-resistant; ESBL=extended spectrum β-lactamase; KPC=*K. pneumoniae* carbapenemase. **Bold** = CDC "Biggest Threats";[78] *Clinical isolates, R.M. Alden Research Laboratory.

	Microbe	**% CFU Reduction**
Gram positive	*S. aureus*	99.99%
	MRSA*	99.97%
	Vancomycin-resistant *E. faecium* (VRE)*	99.94%
	S. pyogenes	99.35%
Gram negative	*A. baumannii**	100%
	MDR A. *baumannii**	100%
	ESBL *E. coli**	100%
	K. pneumoniae	100%
	ESBL, KPC *K. pneumoniae**	100%
	P. aeruginosa	100%
	MDR *P. aeruginosa**	100%
Anaerobe	MDR *B. fragilis**	99.77%
Fungi	*C. albicans*	100%
	Fluconazole-resistant *C. albicans**	100%

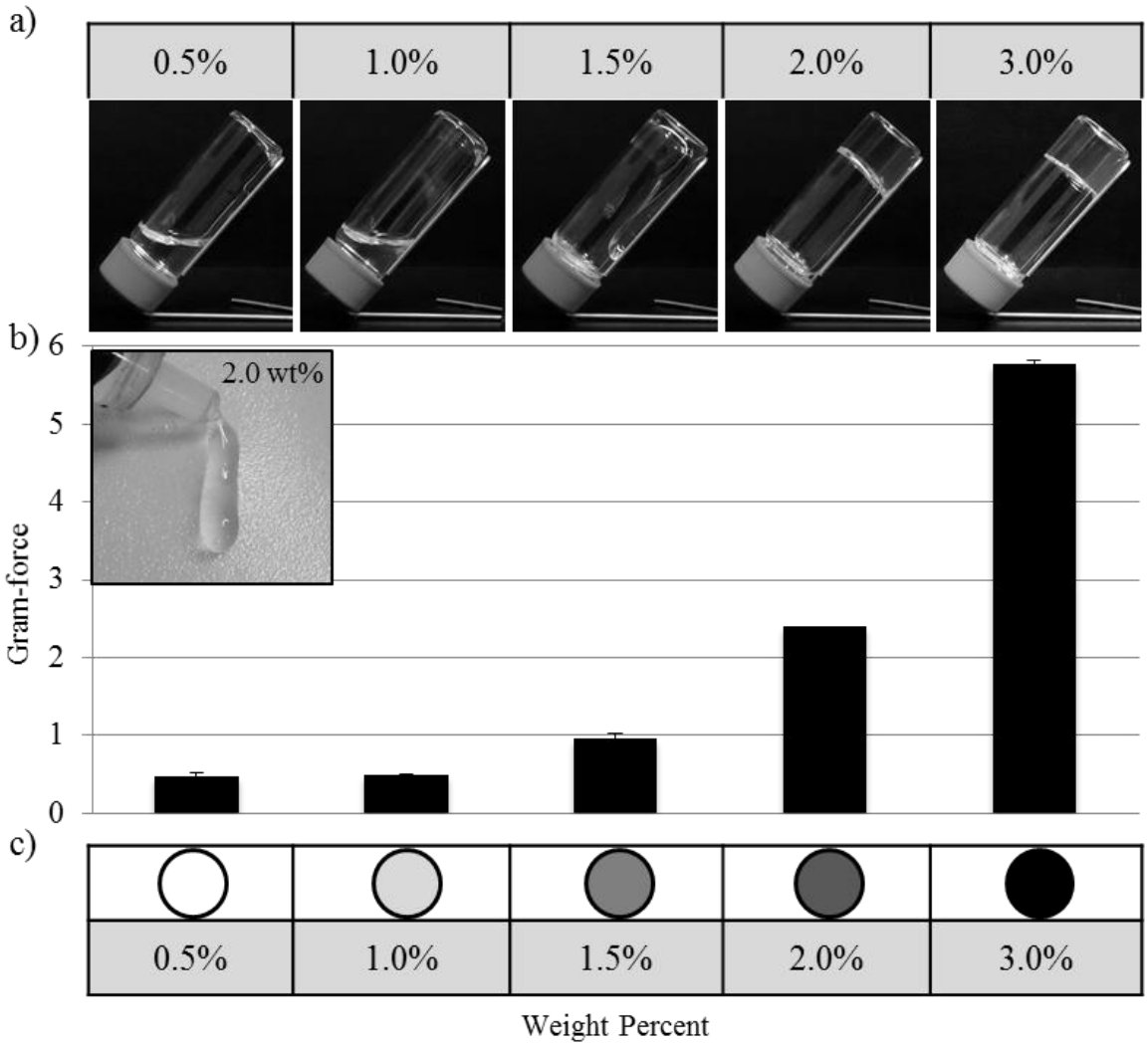

Figure 3. $K_{100}L_{40}$ block copolymers form hydrogels in water. $K_{100}L_{40}$ copolymers were prepared in DI water at concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0 wt% and assessed for gel formation by tilt tube assay, firmness by texture analysis, and viscosity. (a) Visual gelation study by tilt tube assay. (b) Concentration-dependent firmness in water as measured by texture analysis. Firmness values were taken at a probe depth of 8 mm. Inset picture depicts 2 wt% $K_{100}L_{40}$ in water applied to an artificial skin substrate (VITRO-SKIN; IMS Inc.). (c) Graphical representation showing change in physical properties with increasing concentration of $K_{100}L_{40}$ copolymers (white = fluid; black = firm gel); based on data from viscometry for 0.5 and 1.0 wt% and data from texture analysis and from tilt tube assay for the higher concentrations. Data were obtained from triplicate measurements in a single experiment and are presented as mean + SD.

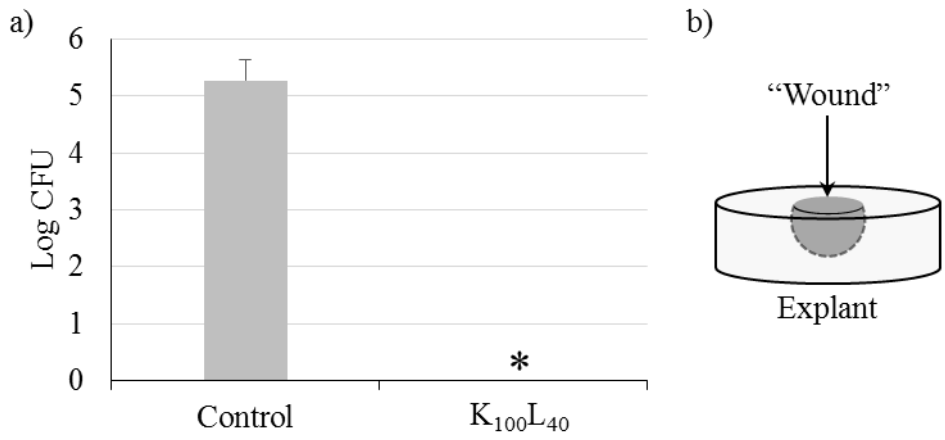

Figure 4. Antimicrobial barrier properties of $K_{100}L_{40}$ preparations demonstrated on porcine skin ex vivo. (a) Data show log CFU of surviving *P. aeruginosa* 3 hours after inoculation of skin explants that were pretreated with water (control; N=8) or pretreated with 1 wt% $K_{100}L_{40}$ (N=8); $p<0.0001$. Data are presented as mean + SD. (b) Schematic of porcine explant depicting artificial wound. * = no microbes detected.

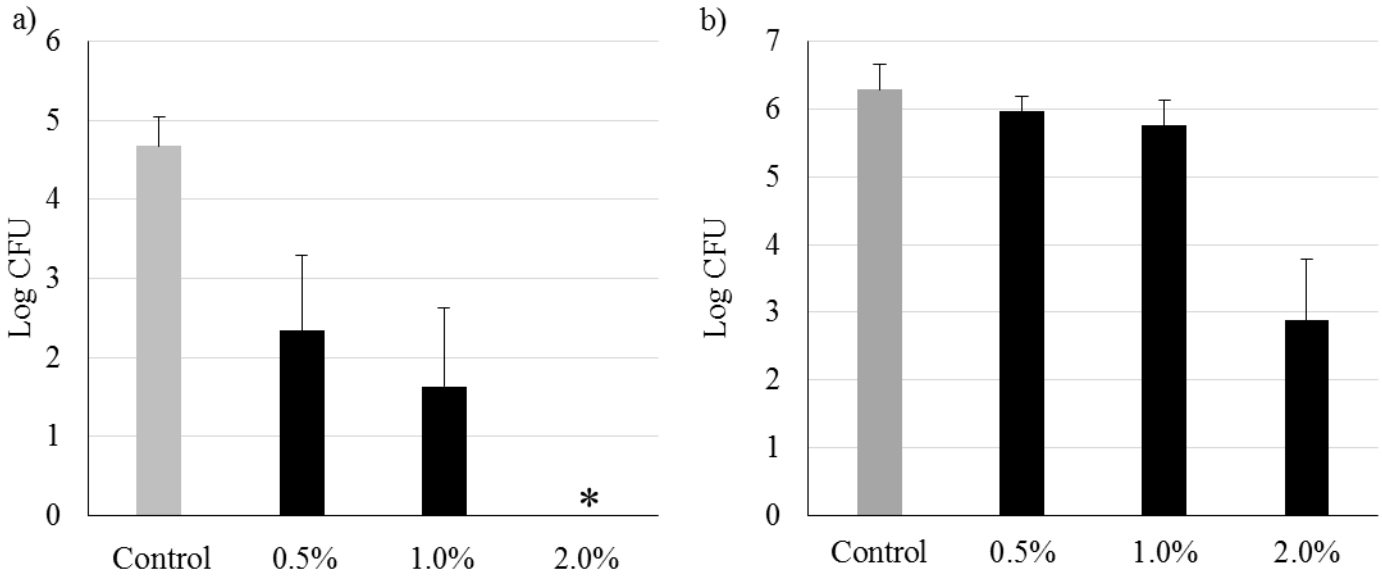

Figure 5. $K_{100}L_{40}$ preparations were found to prevent microbial contamination in a porcine open wound model (N = 5 wounds per group; 2 biopsies per wound). (a) *S. epidermidis,* (b) *P. aeruginosa*. Full-thickness wounds were pretreated with 1.0 mL of $K_{100}L_{40}$ or control (water) 15 minutes prior to inoculation with bacteria. Wounds were then biopsied for microbial counts after 4 hours. In both cases, the difference between control and 2% was significant at $p<0.01$. Data are presented as mean + SEM. *No microbes detected.

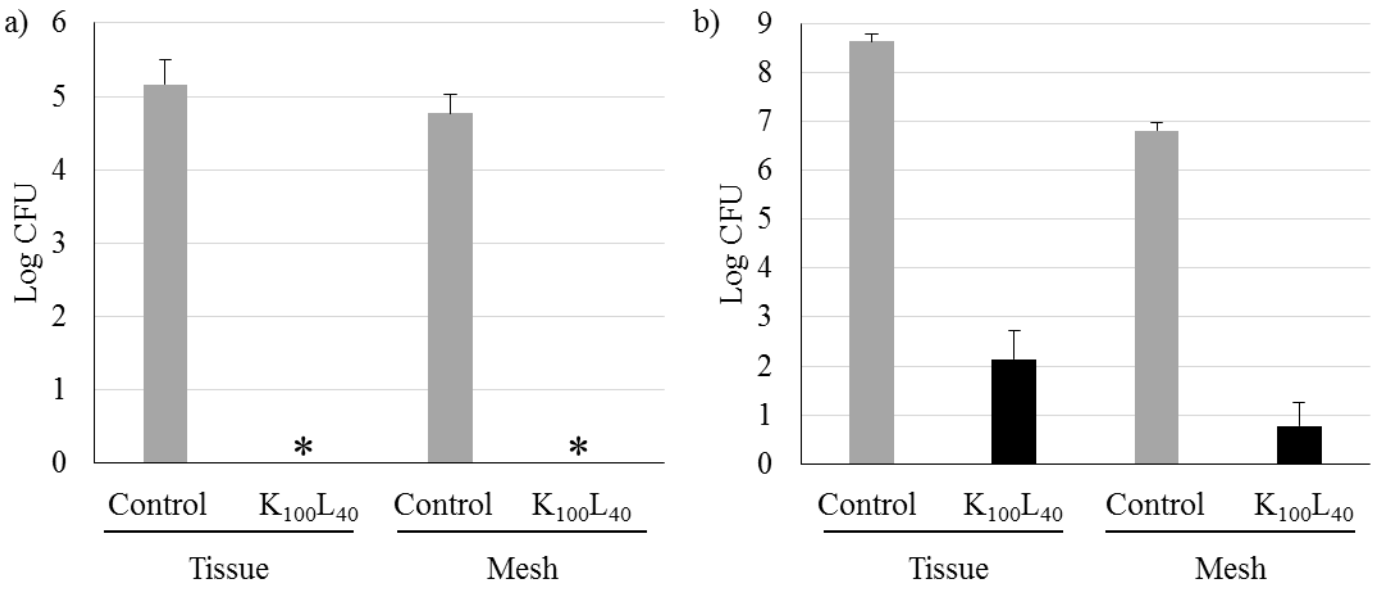

Figure 6. $K_{100}L_{40}$ preparations (1 wt% in water) show activity against (a) MRSA (ATCC 33593) and (b) *P. aeruginosa* (ATCC 27317) in a rodent closed-wound model with foreign body ($K_{100}L_{40}$ N=6; Control N=8; Sprague-Dawley rats). Log CFU survival shown per gram tissue for biopsy samples and per implanted polypropylene mesh. $K_{100}L_{40}$ preparations were applied 15 minutes prior to microbial inoculation; microbial burden was assessed after 48 hours. Differences between control and $K_{100}L_{40}$ groups for both microbes was significant at $p<0.0001$. Data are presented as mean + SEM. *No microbes detected.

Supporting Information

for *Macromol. Biosci.*, DOI: 10.1002/((please add journal code and manuscript number))

Amino Acid Block Copolymers with Broad Antimicrobial Activity and Barrier Properties

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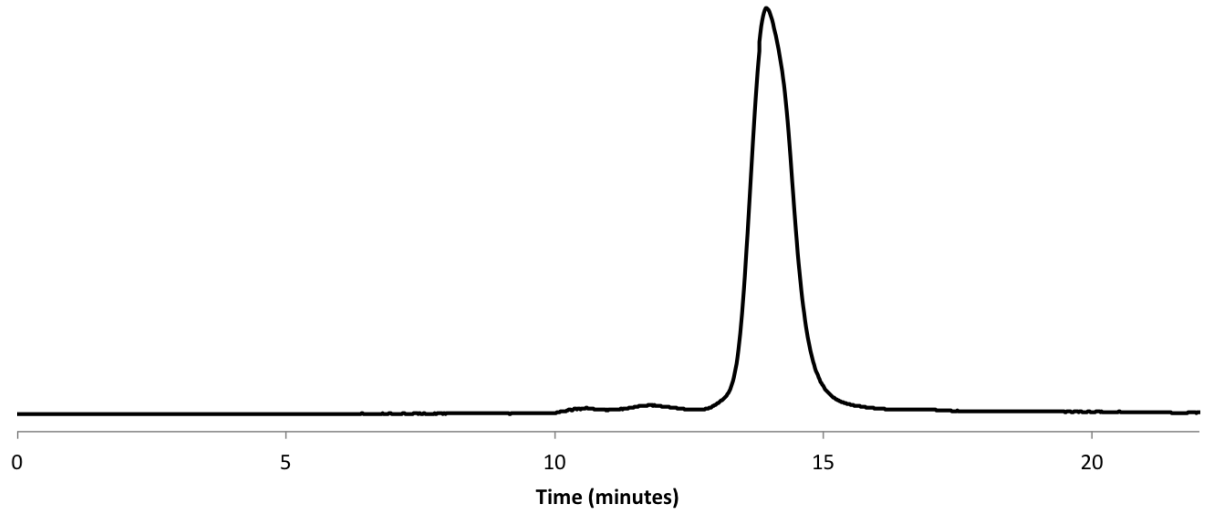

Figure S1: GPC chromatogram showing light scattering intensity of $K_{100}L_{40}$.

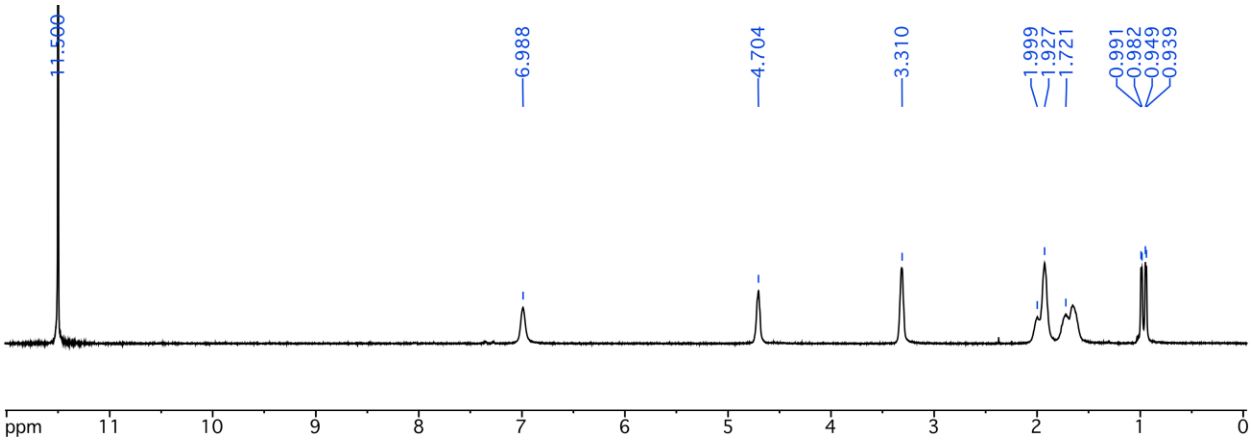

Figure S2: ^{1}H NMR (d-TFA) of $K_{100}L_{40}$.