Designed Host Defense Peptide as Anti-infectives for Treatment of

Acne,
Acute Bacterial Skin and Skin Structure Infections,
Keratitis, &
Recurrent vulvovaginal candidiasis

Riptide Bioscience, Inc.

INFECTIOUS DISEASES Virtual Partnering

2-4 December 2020

Riptide: developing engineered peptide therapeutics

Key scientific leadership:

- Dr. Jesse Jaynes, CSO (dAMP progenitor & professor of Biochemistry)
- Dr. Kathryn Woodburn, VP – Translational Research (experienced in Regulatory Pharm/Tox)
- Dr L. Edward Clemens, Principal Scientist (cross functional drug development expert)
- Dr. George Martin, President (former scientific head of NIA)

Peer-reviewed competitive grant support: $2.8M, DoD and NIH

Riptide: background in dHDPs

Origin of Riptide's drug candidates: research by Prof. Jaynes

- Discovery of structural motifs governing efficacy; extensive screening, sequence modification, iteration & optimization

Program has accelerated with support from Department of Defense

- Acknowledged as a 2018 Defense Medical Research and Development program by CDMRP (https://cdmrp.army.mil/dmrdp/research_highlights/18clemens_highlight)

SBIR support:

- National Eye Institute (keratitis),
- National Institute of Musculoskeletal and Skin Diseases (acne), and
- National Institute of Diabetes and Digestive and Kidney Diseases (diabetic ulcers)

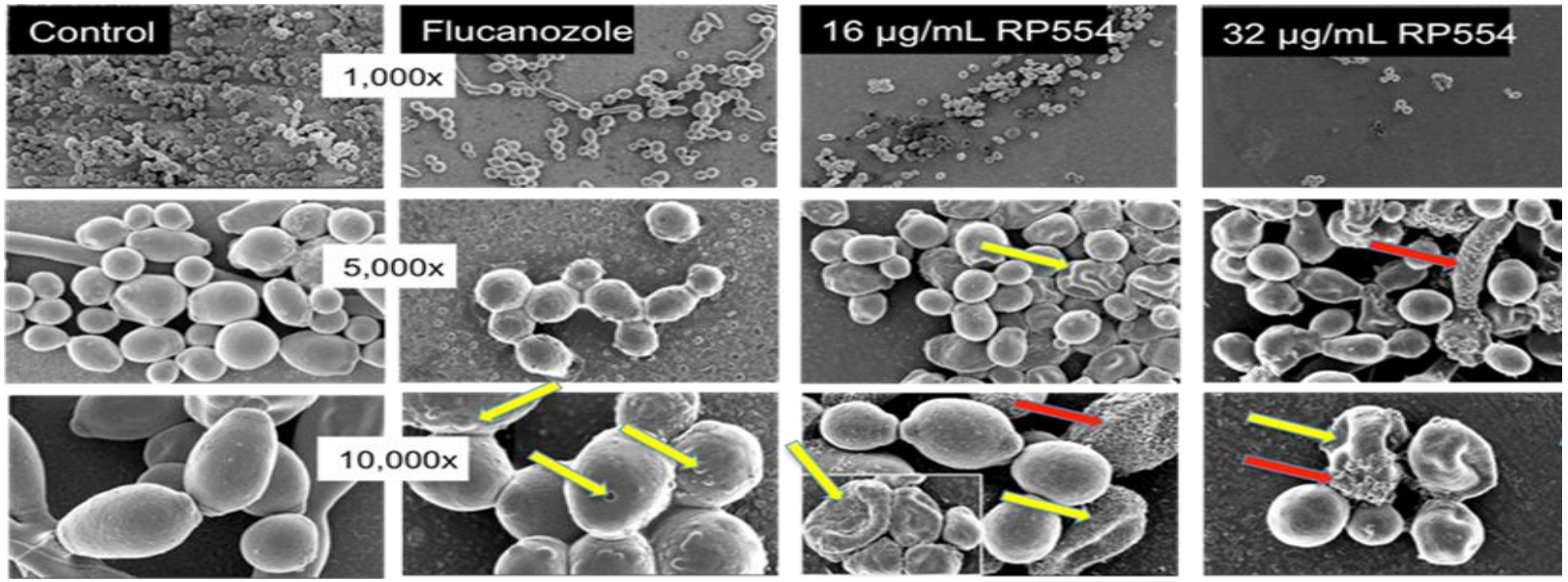
dHDPs: Potential to treat, and/or prevent infections

- Broad spectrum antimicrobial activity allowing empiric treatment,
  - Bacteria
  - Fungus
  - Mycobacteria
- Active against multidrug resistant organisms,
- Rapid antimicrobial effect,
- Less prone to pathogen resistance development,
- Able to prevent biofilm formation,
- Effective against established biofilm,
- High Therapeutic Index,
- Accelerates wound healing, &
- Suppresses inflammatory responses.

dHDPs mechanism of action

Host Defense peptides are the body's "first responders" to microbial invasion

- Initial activity is lytic: strong positive charge attracts AMPs to microbial cell surface – form pore, lyse the cell
- This mechanism is far less vulnerable to bacterial resistance
- Secondary mechanism: mobilize immune system



Woodburn et al., (2019) Designed Antimicrobial Peptides for Recurrent Vulvovaginal Candidiasis Treatment. Antimicrob Agents Chemother. AAC.02690-18.

Broad spectrum antimicrobial activity allowing empiric treatment

Organism	Test Agent	µg/mL				%S	%R
		MIC Range	MIC_{50}	MIC_{90}	Breakpoints*		
P. aeruginosa	RP557	0.25-4	1	1	–	–	–
	Colistin	0.25 - >16	0.25	0.5	≤2 : ≥4	95%	5%
	Levofloxacin	0.25 - >16	>16	>16	≤1 : ≥4	20%	80%
	Meropenem	0.25 - >16	16	>16	≤2 : ≥8	20%	80%
	Tobramycin	0.25- >16	8	>16	≤4 : ≥16	40%	50%
A. Baumannii	RP557	0.5 - 4	1	2	–	–	–
	Colistin	0.06 - >16	0.25	4	≤2 : ≥4	80%	20%
	Levofloxacin	0.06 - >16	16	>16	≤0.5 : >1	5%	90%
	Meropenem	0.5 - >16	>16	>16	≤2 : ≥8	5%	95%
	Tobramycin	0.25 - >16	>16	>16	≤4 : >4	30%	60%

Studies performed by Micromyx LLC, Kalamazoo, MI. against *Pseudomonas aeruginosa* (n=20) and *Acinetobacter baumannii* (n=20), consisting primarily of carbapenem-resistant clinical isolates.

*Breakpoints: Sensitive (S) : Resistant (R).

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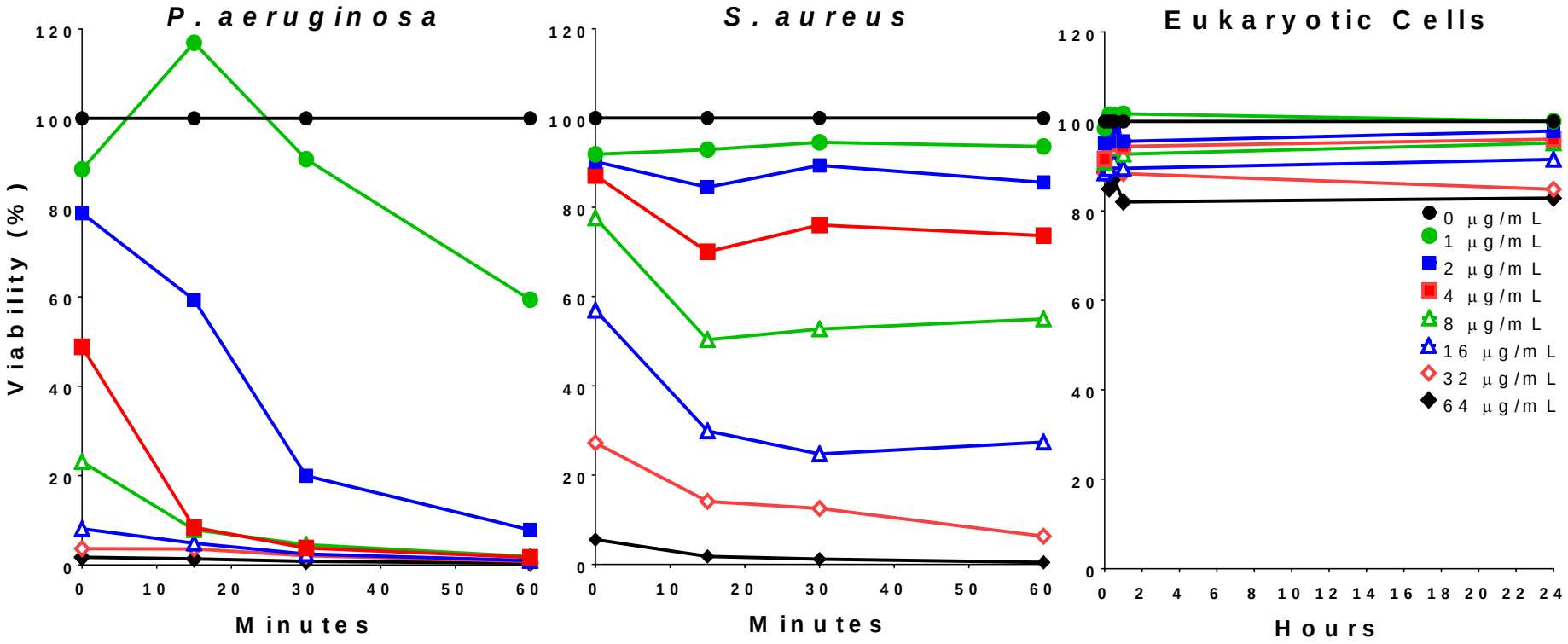
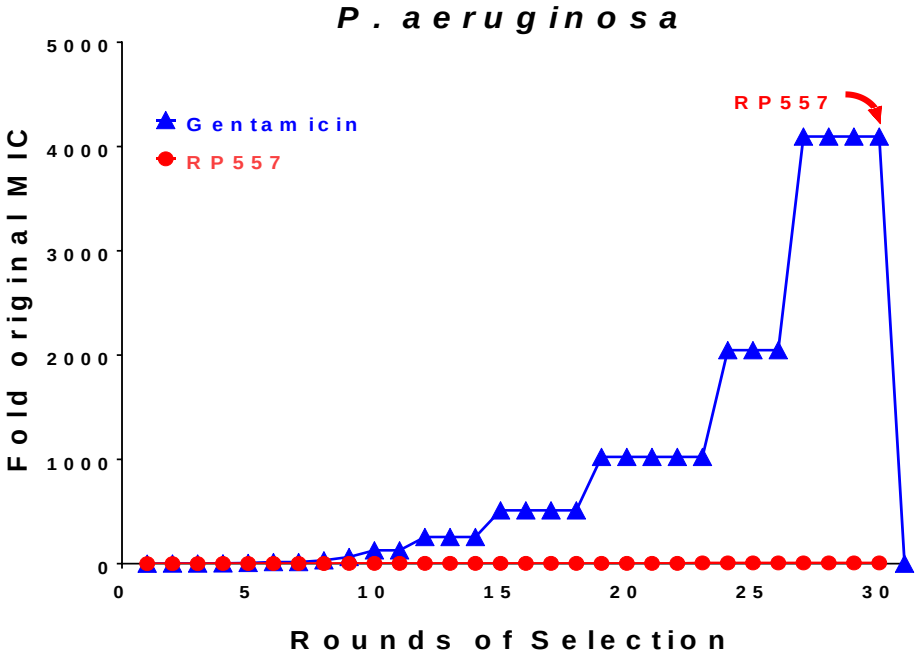
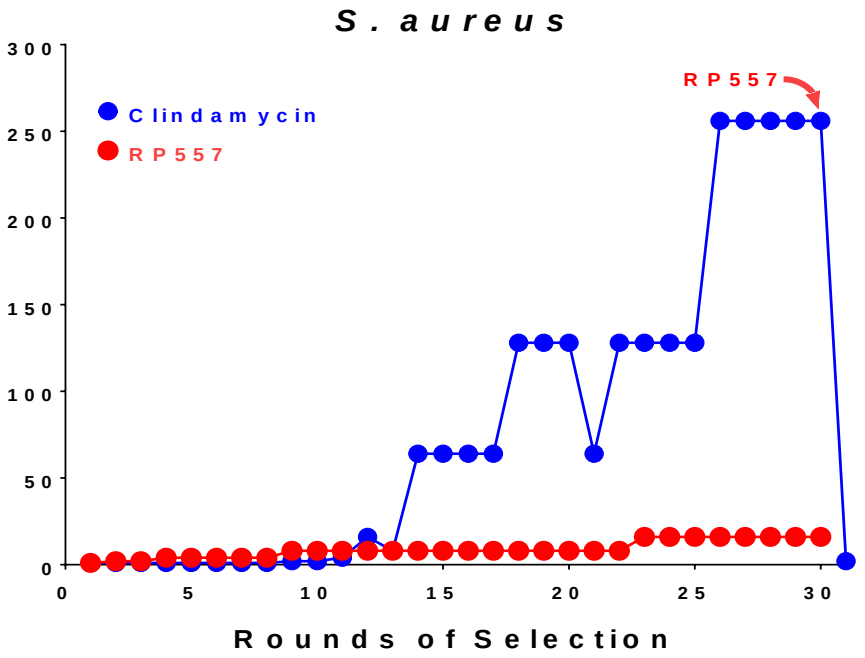
Rapid antimicrobial effect



RP557 rapidly eradicates *P. aeruginosa* and *S. aureus* with no cytotoxicity to mammalian cells. Cell viability was performed using bioluminescent strains.

Less prone to pathogen resistance development

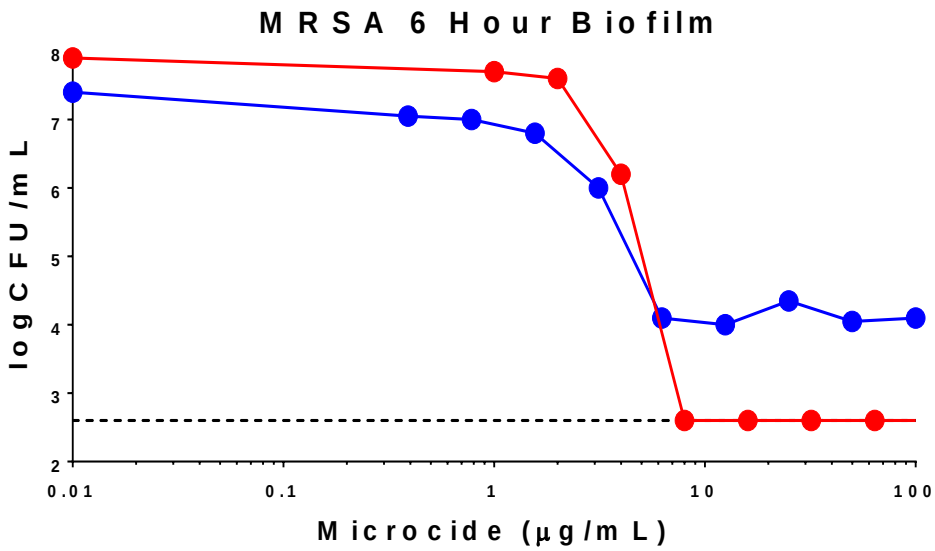


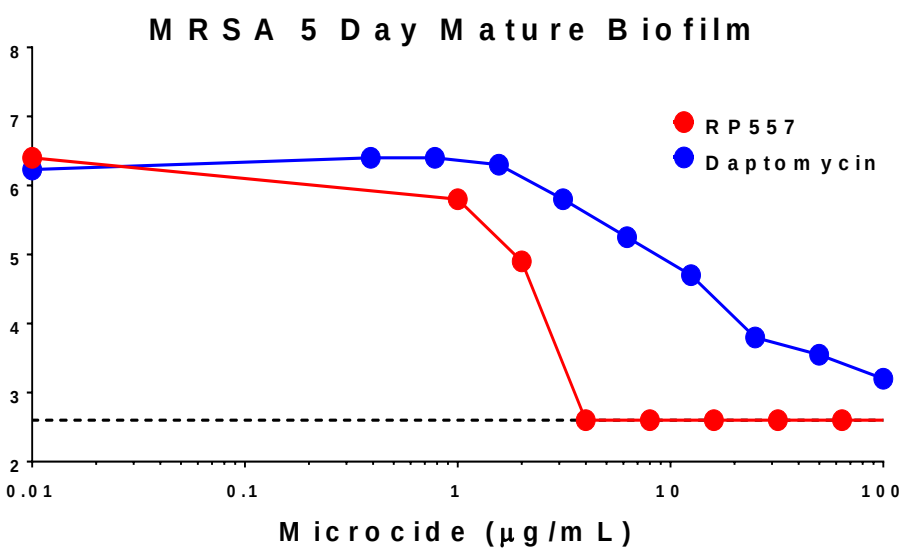


Pathogens did not develop resistance against RP557. Sub-inhibitory concentrations of RP557, gentamicin and clindamycin with *P. aeruginosa* 27853 and *S. aureus* 29213 for 24 hours. Bacteria showing growth in the highest concentration were re-passaged in fresh dilutions containing sub-MIC levels of each component for 30 consecutive passages; means are shown.

Woodburn et al. (2019) Evaluation of the Antimicrobial Peptide, RP557, for the Broad-Spectrum Treatment of Wound Pathogens and Biofilm· Front Microbiol. Jul 24;10:1688.

Able to prevent and treat established biofilm



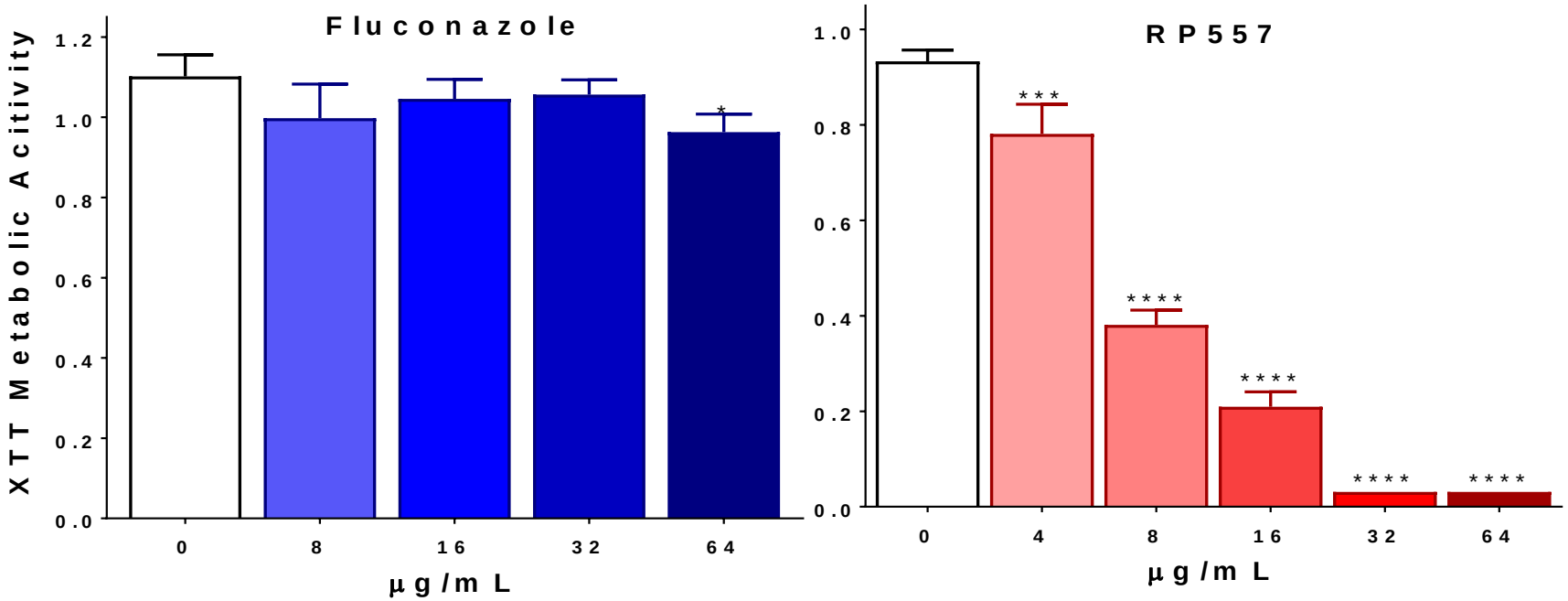


RP557 completely eradicated both preformed and mature MRSA biofilm exhibiting EC_{50s} of 4.21 and 2.12 µg/mL, respectively. In contrast, daptomycin was only able to reduce preformed MRSA biofilm by 55% yielding an EC_{50} of 4.1 µg/mL however the concentration required to reduce mature biofilm by 50% was exponentially larger at approximately 100 µg/mL.

Woodburn et al. (2019) Evaluation of the Antimicrobial Peptide, RP557, for the Broad-Spectrum Treatment of Wound Pathogens and Biofilm. Front Microbiol. Jul 24;10:1688.

Fungal biofilm eradication

Biofilm formation is a key driver of fungal pathogenicity.



RP557 effectively kills preformed *C. albicans* biofilm and prevents biofilm formation (not shown) while fluconazole is ineffective. Data represent the mean±SD of triplicate measurements and statistical significance, compared to vehicle control, determined by one-way ANOVA followed by Dunnett's test (*p < 0.05, ***p < 0.001, and ****p < 0.0001).

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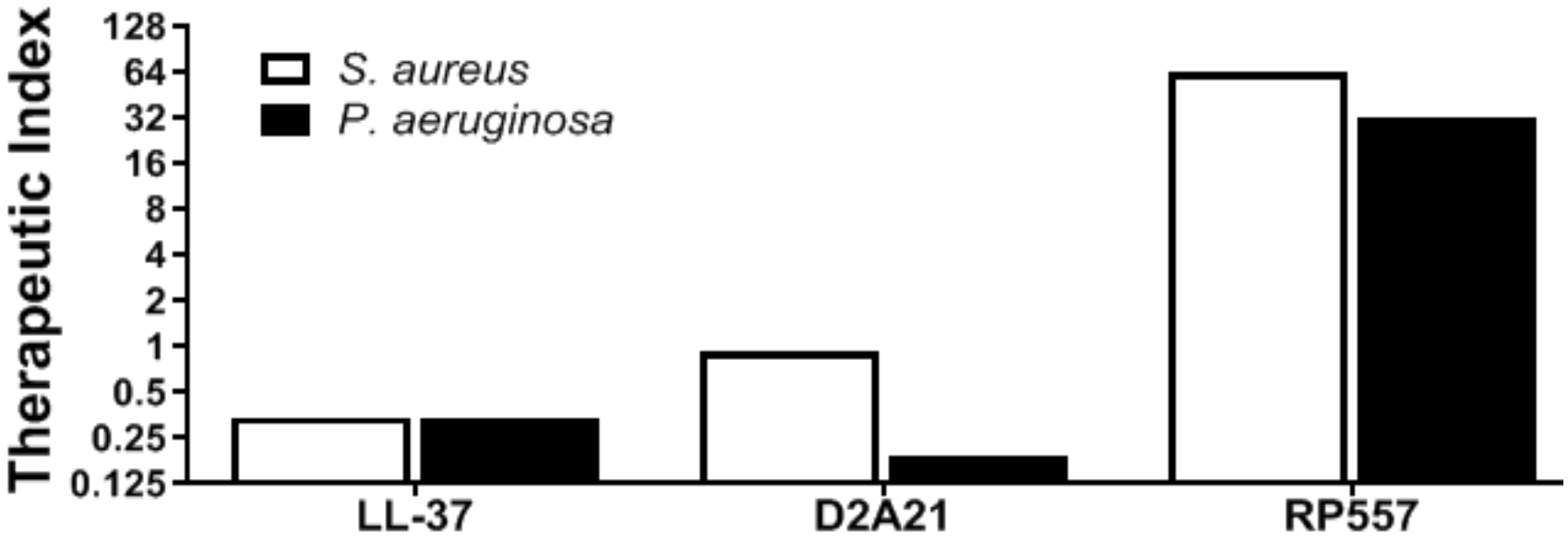
High Therapeutic Index



Therapeutic Index is defined by the dose required to hemolyze 10% (EC_{10}) human red blood cells compared to the *S. aureus and P. aeruginosa* minimal inhibition concentration (MIC).

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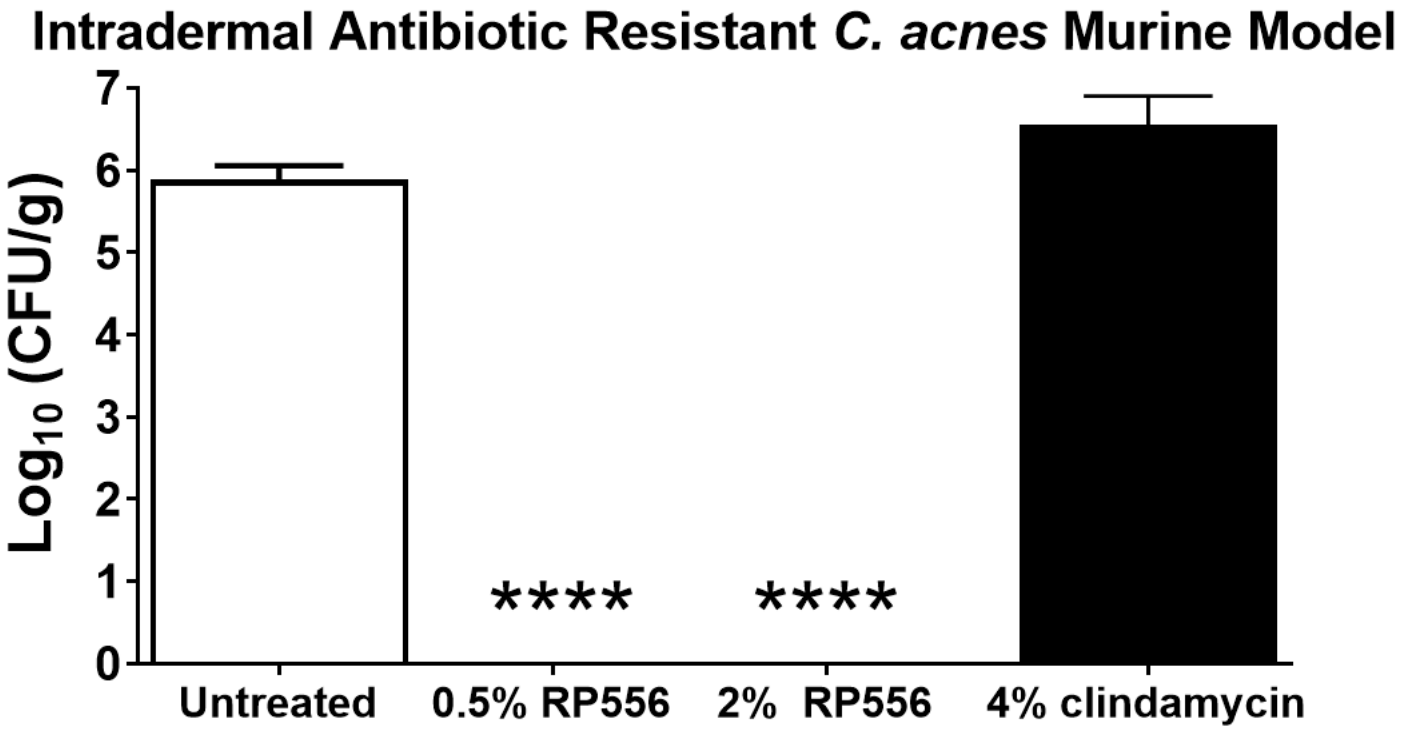
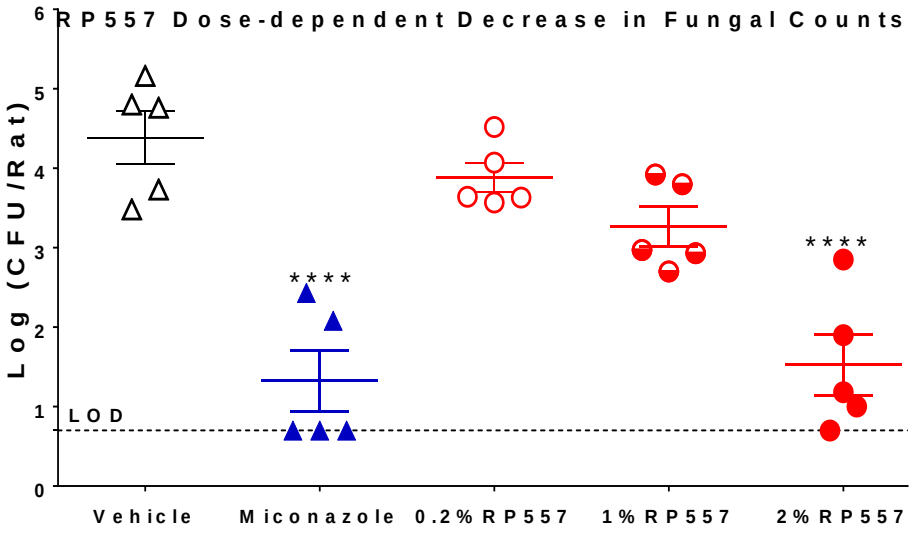
Acne infection elimination

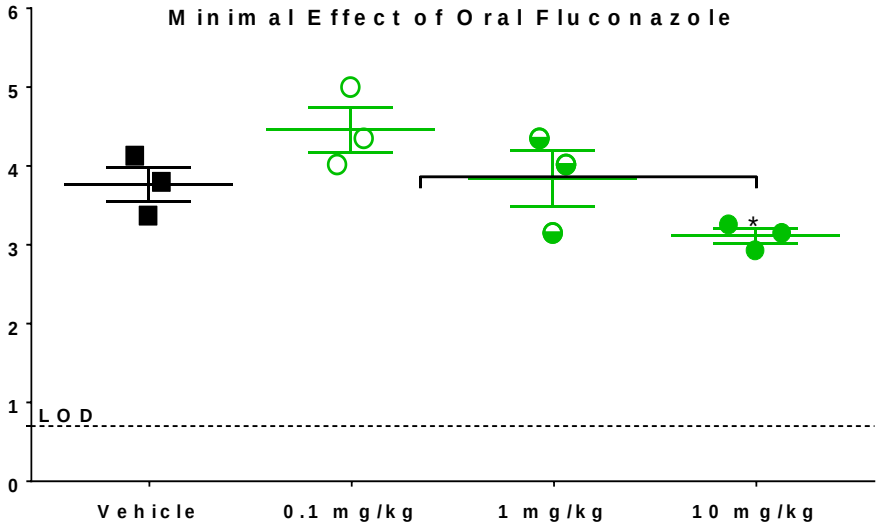


RP556 or clindamycin, was applied topically at 2, 14, 26, 38, 50, 62 &72 hours post infection to Balb/c mice. Skin was harvested at 96 hours for bacterial concentrations (CFU/gram tissue). N= 6 measurements for untreated and RP556 groups and n=4 for the clindamycin group. (****, P <0.0001) by one-way ANOVA, RP556 vs Untreated and clindamycin treated groups.

Woodburn KW et al. Designed Antimicrobial Peptides for Topical Treatment of Antibiotic Resistant Acne Vulgaris. Antibiotics 2020;9(1):23.

Treatment of recurrent vulvovaginal candidiasis



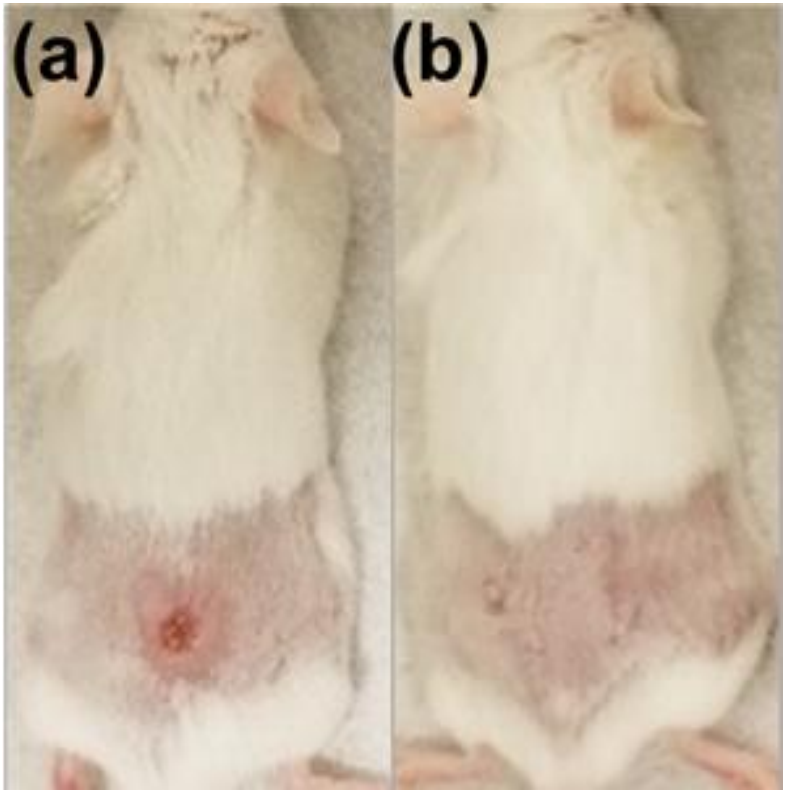


RP557 was effective in eradicating *C. albicans* in a rodent model of vulvovaginal candidiasis. On Day 0, rats were inoculated intravaginally with *C. albicans* at 1.46 x 10^7 CFU/rat. RP557 and 2% miconazole were administered twice daily at 8 hr intervals starting from 48 hr after infection for 3 days. CFUs were evaluated on Day 5 with LOD of 0.7 CFU/rat. Significant difference, compared to vehicle, determined by one-way ANOVA followed by Dunnett's test (*$p < 0.05$, ****$p < 0.0001$). A separate group receiving oral fluconazole; *$p < 0.05$ versus 0.1 and 10 mg/kg fluconazole.

Woodburn et al., (2019) Designed Antimicrobial Peptides for Recurrent Vulvovaginal Candidiasis Treatment. Antimicrob Agents Chemother. AAC.02690-18.

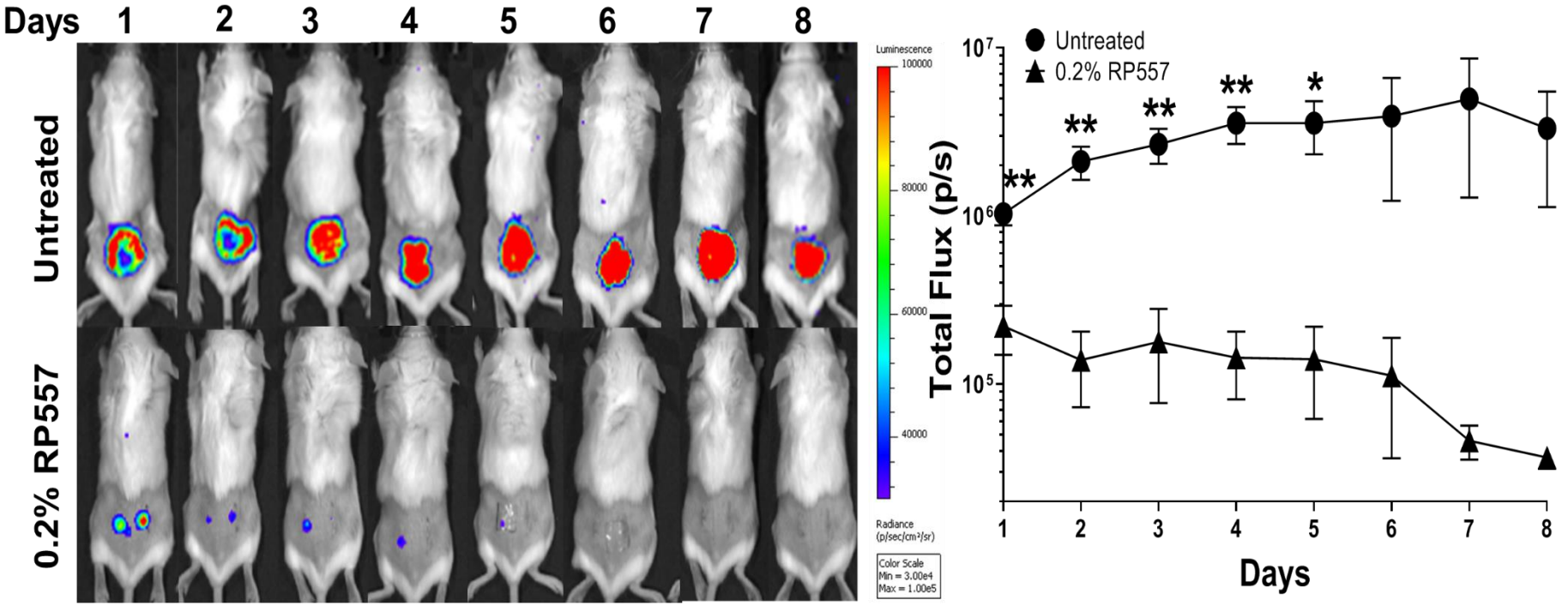
Accelerates wound healing

Topical RP557 treatment enhances wound healing in a murine MRSA infected abrasion wound model



BALB/c mice were IP administered cyclophosphamide 4 days and 1 day prior to MRSA inoculation. Skin abrasion wounds were introduced on the shaved dorsal surface of Balb/c mice. Five minutes following wounding, 40 µL of 10^8 CFU/mL bioluminescent MRSA (Xen 31) in PBS, was inoculated over the wounded scratched area producing a total inoculum of 4×1^06 CFU. Representative photographs of the backs of the mice on Day 13 are shown with a Vehicle treated animal in (a) and an RP557 treated animal in (b). By Day 13, RP557 treatment had completely healed the wound lesion whereas the Vehicle control animal had still possessed a pronounced wound.

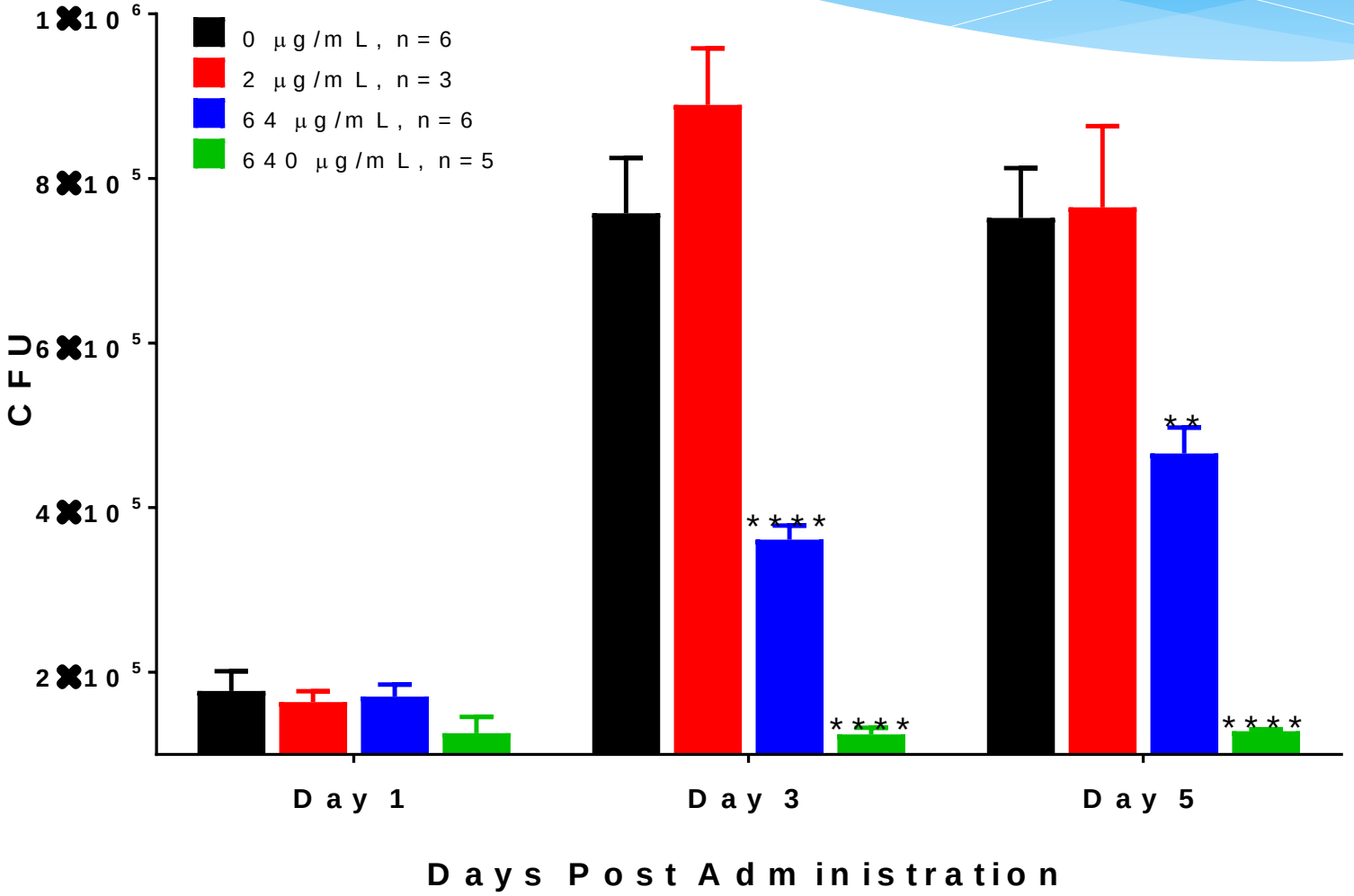
Treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI)



A single topical treatment of RP557 eradicates MRSA. Infected scratch wounds were created on the backs of immunosuppressed BALB/c mice with bioluminescent MRSA (Xen31, MRSA ATCC 33591). After 4 hours, 0.2% RP557 in 2% hydroxypropyl methylcellulose was applied. Data is expressed as mean±SE of 5 mice. Statistical significance, compared to untreated control, determined by two-tailed unpaired-t test (*$p < 0.05$, **$p < 0.01$).

Woodburn et al. (2019) Evaluation of the Antimicrobial Peptide, RP557, for the Broad-Spectrum Treatment of Wound Pathogens and Biofilm. Front Microbiol. Jul 24;10:1688.

RP444 reduces bacterial burden in a *P. aeruginosa* keratitis model

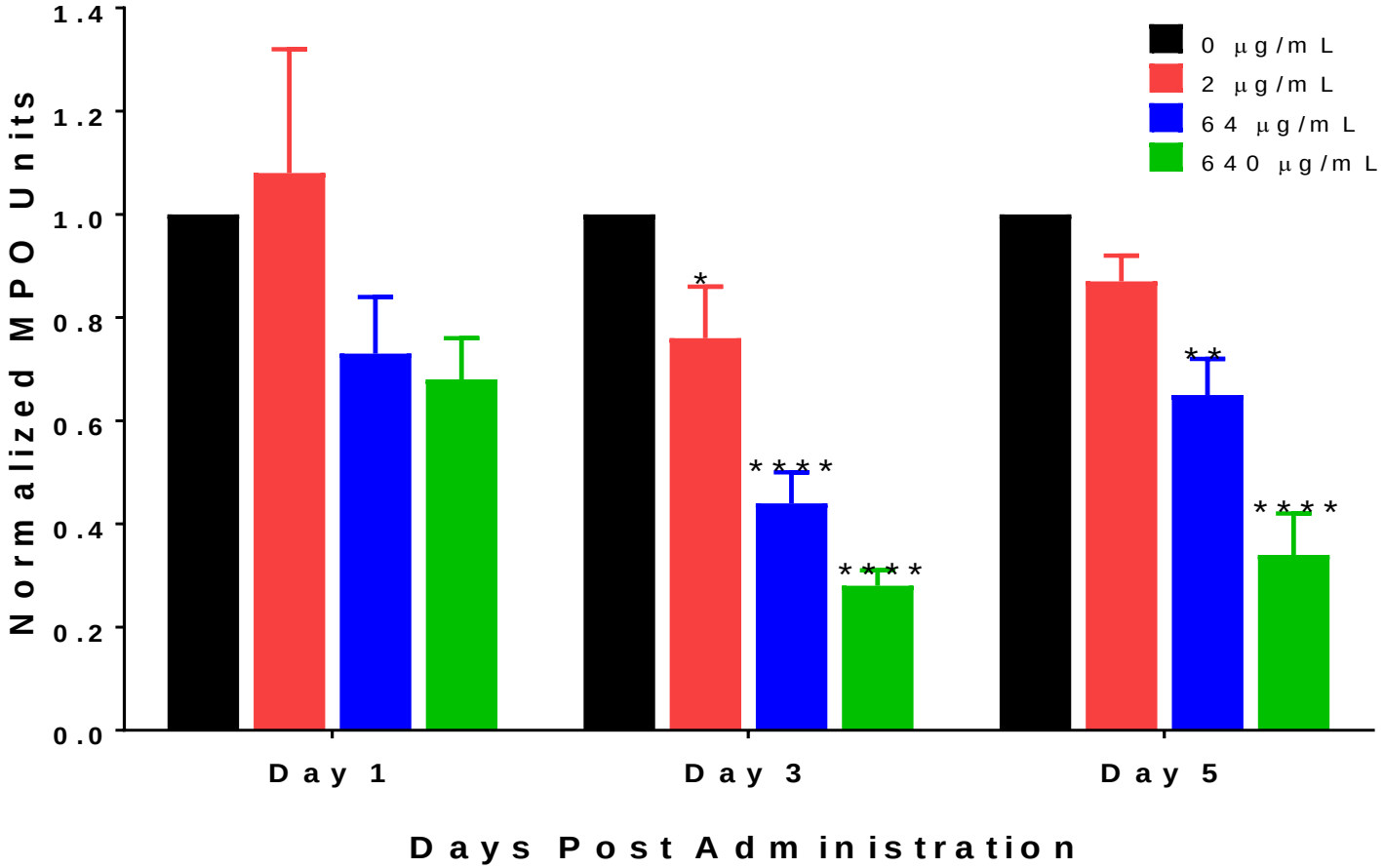


*p < 0.5, **p < 0.01, & ***p < 0.001 compared to PBS treated control

Clemens et al (2017). Designed Host Defense Peptides for the Treatment of Bacterial Keratitis. Invest Ophthalmol Vis Sci58, 6273-6281.

Suppresses inflammatory responses

RP444 reduces inflammatory cell infiltration in a *P. aeruginosa* murine keratitis model



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