Supplementary Information

Nucleic acid peptide nanogels for the treatment of bacterial keratitis

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Results

Table S1: Sequences of ssDNA designed for the fabrication of DNA nanogels

	Name	Sequences	M_w (g/mole)	$^{\alpha}$Tm (°C)	$^{\beta}$GC content (%)
YMA	Y_1	5'- GAG TTG GGT CTC GAG TTC CTC CGA GGT CAT TCT CGA CAA G -3'	12,319.0	67.5	55.0
	Y_2	5'- GAG TTG GGT CTC CAG TAC TAT GCT CAC CTC GGA GGA ACT C -3'	12,288.0	67.2	55.5
	Y_3	5'- GAG TTG GGT CTC CTT GTC GAG AAT GAT GAG CAT AGT ACT G -3'	12,422.1	64.4	47.5
YMB	Y_1	5'- GAG TTG GGT CTC GAG TTC CTC CGA GGT CAT TCT CGA CAA G -3'	12,319.0	67.5	55.0
	$Y_{2\,(NS)}$	5'- GAG TAC TAT GCT CAC CTC GGA GGA ACT C -3'	8533.6	61.2	53.6
	$Y_{3\,(NS)}$	5'- CTT GTC GAG AAT GAT GAG CAT AGT ACT G -3'	8667.7	56.8	42.9
DL	L_3	5'- GAG ACC CAA CTC ACT AAG ACT GGA CTA CCT GAA GAG ATC ACT GG -3'	13,527.8	66.1	50.0
	L_4	5'- GAG ACC CAA CTC CCA GTG ATC TCT TCA GGT AGT CCA GTC TTA GT -3'	13,442.7	66.8	50.0

$^{\alpha}$ Melting temperatures determined using the IDT OligoAnalyzer 3.1 Software available on https://sg.idtdna.com/calc/analyzer.

$^{\beta}$ GC contents of the sequences is determined using the IDT OligoAnalyzer 3.1 Software available on https://sg.idtdna.com/calc/analyzer.

Table S2: Sequence and characterization of L12 antimicrobial peptide (96.1% Purity)

Amino acid sequence	Peptide denotation	[a] Number of repeat units	Theoretical Mw	[b] Measured Mw	Charge
LKKLLKKLLKKL	LL-12	3	1466.0	1466.0	+6

[a] Repeat unit for the designed peptide is LKKL.

[b] Measured by MALDI-TOF MS.

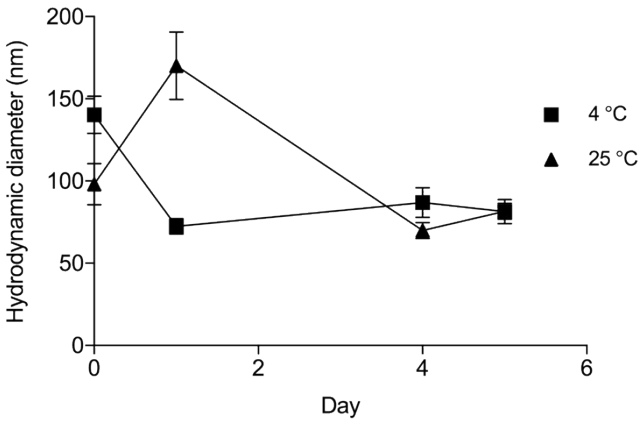

Figure S1: Effect of storage temperature on hydrodynamic diameter of blank nanogels over 5 days.

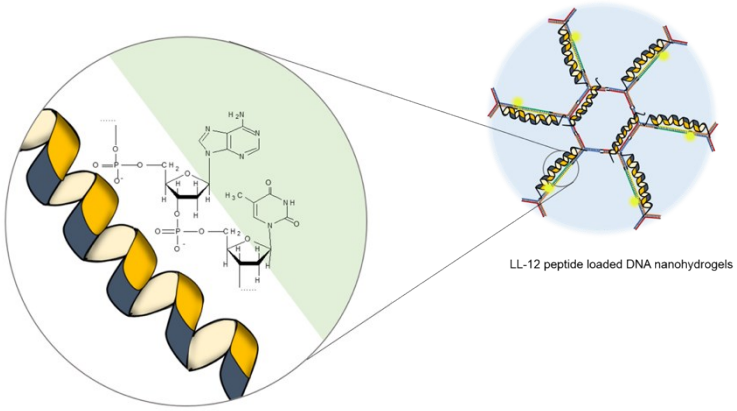

Figure S2: Schematic illustration of non-covalent electrostatic interaction between cationic AMP and anionic phosphate backbone of DNA. (The yellow colored region represents the cationic lysine residues and the dark blue region represents the hydrophobic leucine residues).

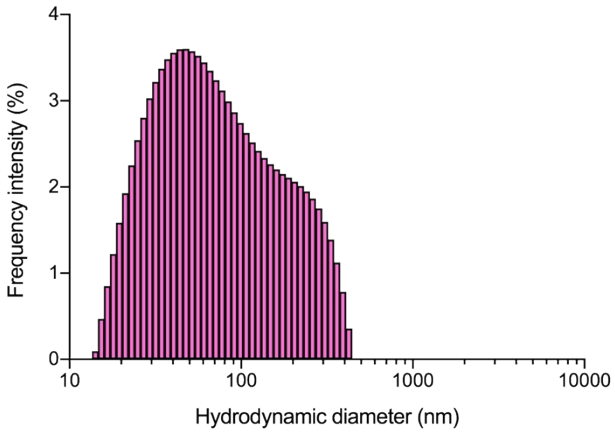

Figure S3: Size distribution of the blank nanogels.

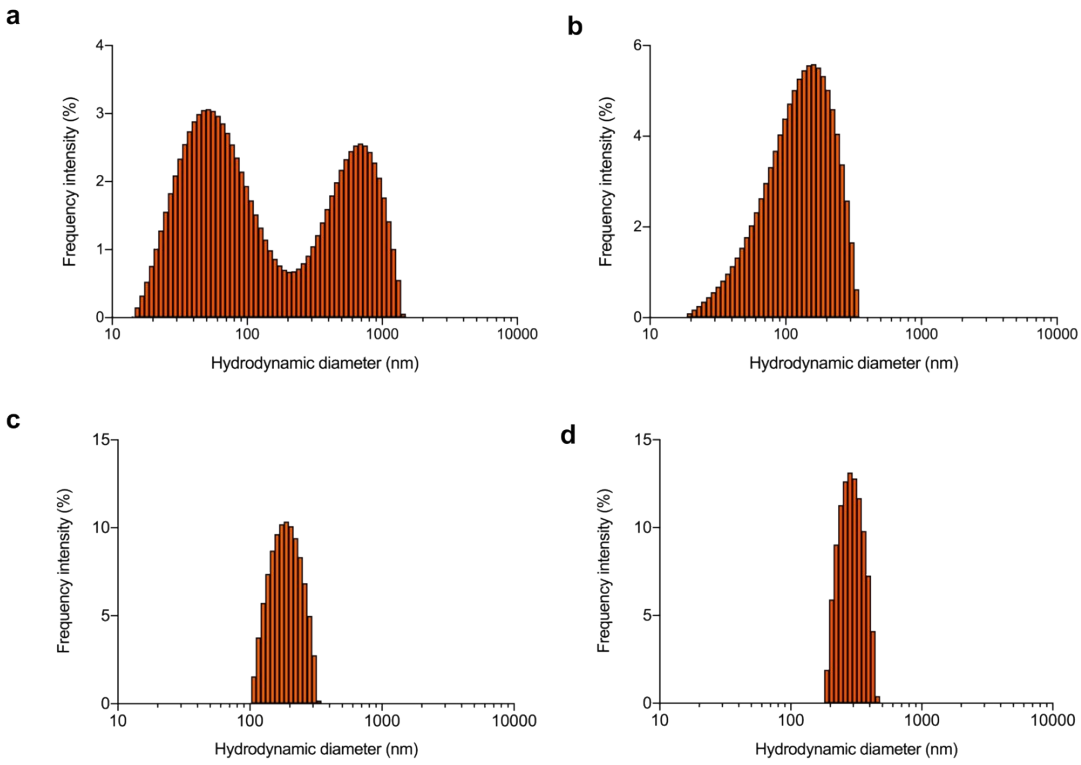

Figure S4: Size distribution of the pre-loaded L12 nanogels **a.** 16 μM L12 nanogels. **b.** 23 μM L12 nanogels. **c.** 32 μM L12 nanogels. **d.** 46 μM L12 nanogels.

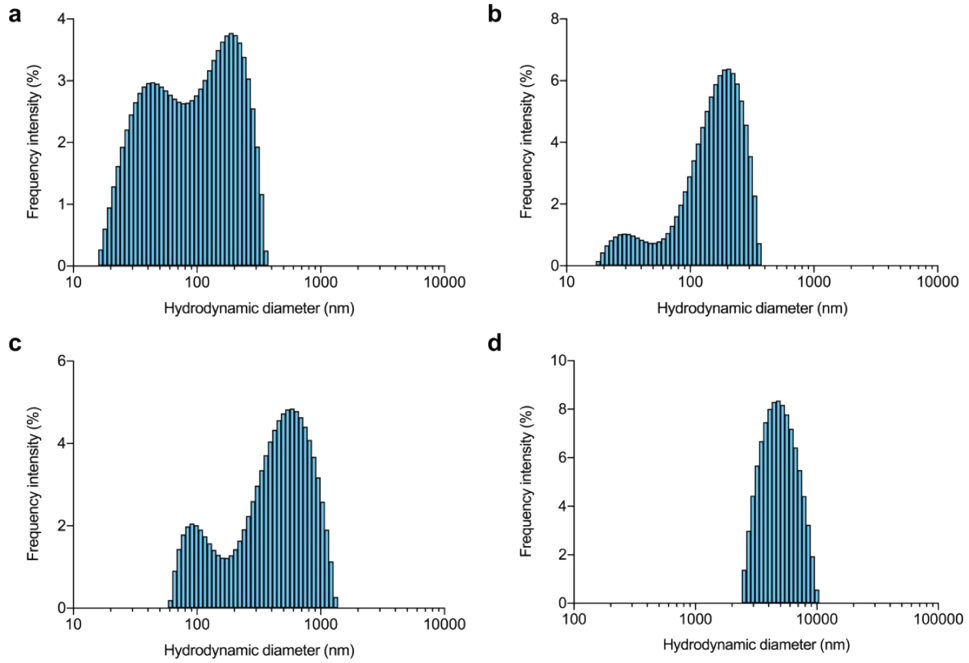

Figure S5: Size distribution of the post-loaded L12 nanogels **a.** 11.5 μM L12 nanogels **b.** 23 μM L12 nanogels **c.** 46 μM L12 nanogels **d.** 92 μM L12 nanogels.

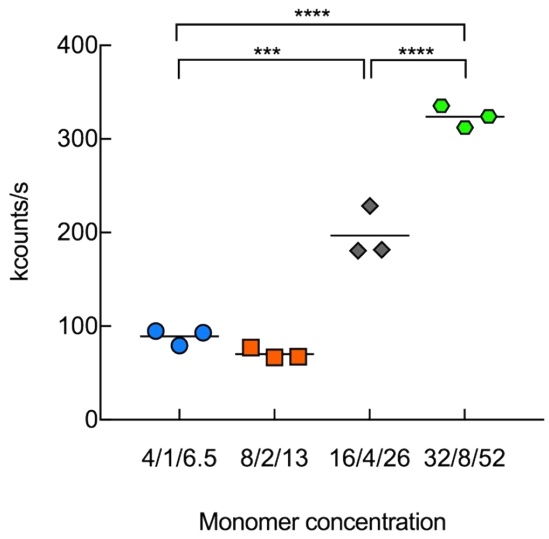

Figure S6: Effect of monomer ratio on kcounts/s of blank nanogels.

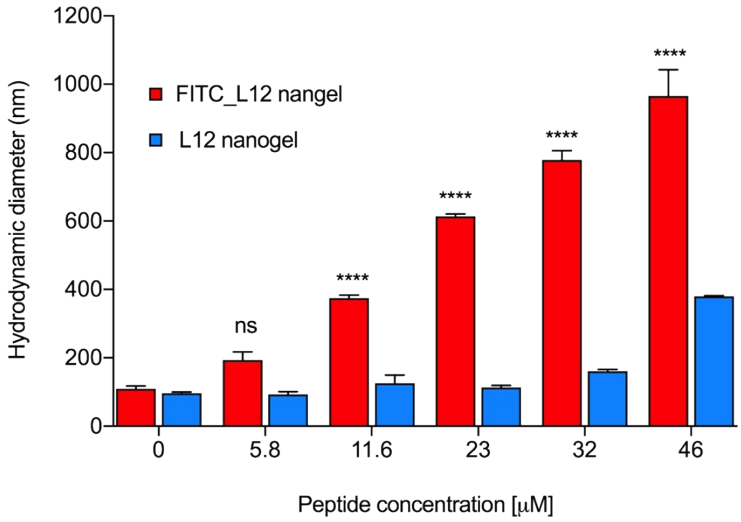

Figure S7: Effect of FITC modification on hydrodynamic diameter of the pre-loaded L12 nanogels.

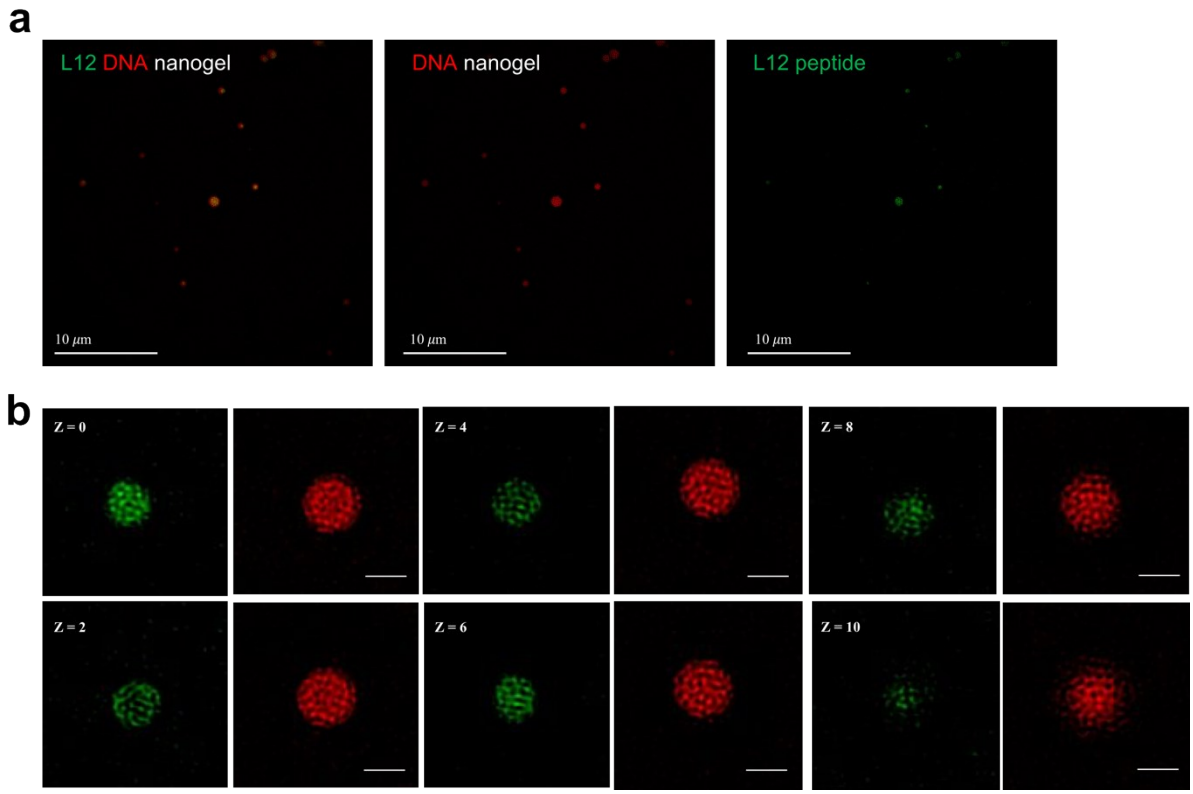

Figure S8: a. Structured illumination microscopy (SIM) images of DNA nanogels containing L12 peptide. DNA nanogels are labelled with Alexa-594 and L12 peptide is labelled with FITC. **b.** SIM confocal Z-stacks (step size: 0.125 μm) images of the L12 nanogels. Individual frames of the green and red channel are shown to demonstrate distribution of L12 within the nanogels.

Table S3: Minimum inhibitory concentration of L12 loaded nanogels

Formulation	*Pseudomonas aeruginosa (ATCC 9027)*	*Staphylococcus aureus (ATCC 29737)*	*Methicillin Resistant Staphylococcus aureus (MRSA)*
L12 Nanogel	11.5 μM	11.5 μM	23 μM

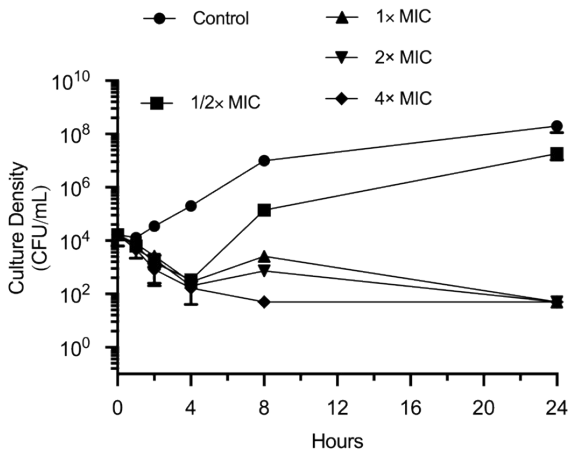

Figure S9: Time kill kinetics of L12 loaded nanogels against *Staphylococcus aureus* (10^5 CFU/mL) over 24h.

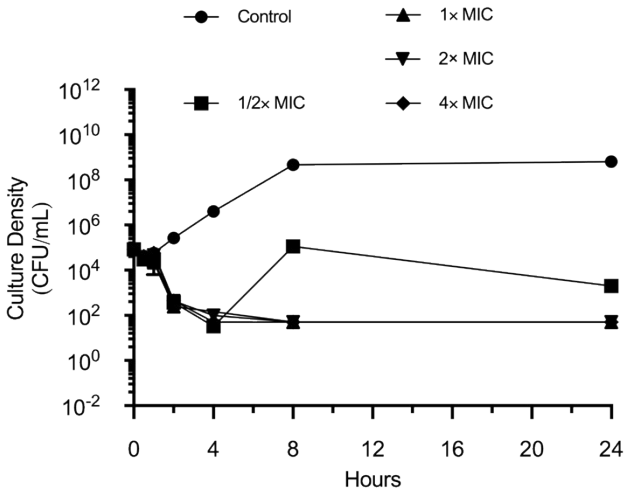

Figure S10: Time kill kinetics of L12 loaded nanogels against *Pseudomonas aeruginosa* (10^5 CFU/mL) over 24h.

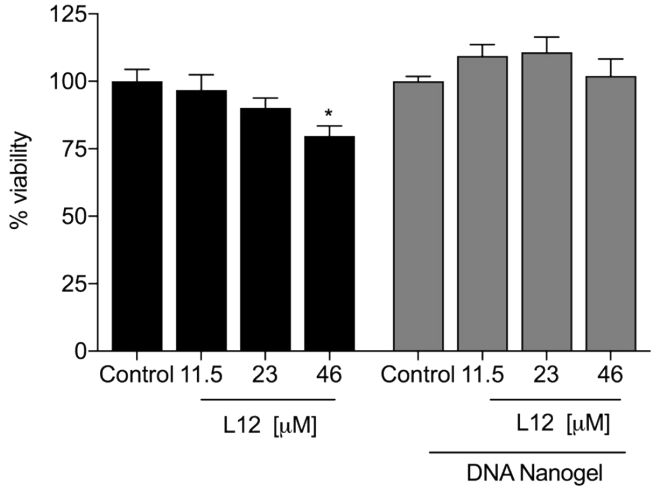

Figure S11: Toxicity of the L12 nanogels against HaCaT cells over 24 h.