Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Effect of Polycation Nanostructures on Cell Membrane Permeability and Toxicity

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1. Determination of the critical micellar concentration (CMC) for PAMAlks.

The CMC value for the polycations was determined using a fluorescent molecular probe technique as described previously.[1] A stock solution of 1,6-diphenyl-1,3,5-hexatriene was prepared in methanol at a concentration of 0.4 mM by short sonication in a bath sonicator. A series of samples containing PAMAlks at various concentrations (0 - 0.1 mg/mL) and a constant DPH concentration (4 μM) were prepared and stirred for 4 hours in the dark. The fluorescence spectra of these samples were measured using the FS5 Spectrofluorometer (Edinburgh Instrument) (**Fig. S1**). The samples were excited at 350 nm and the fluorescence intensity at 440 nm in the presence of PAMAlks was analyzed. Results are presented in **Table 1** and **Fig. 2** and **S2**.

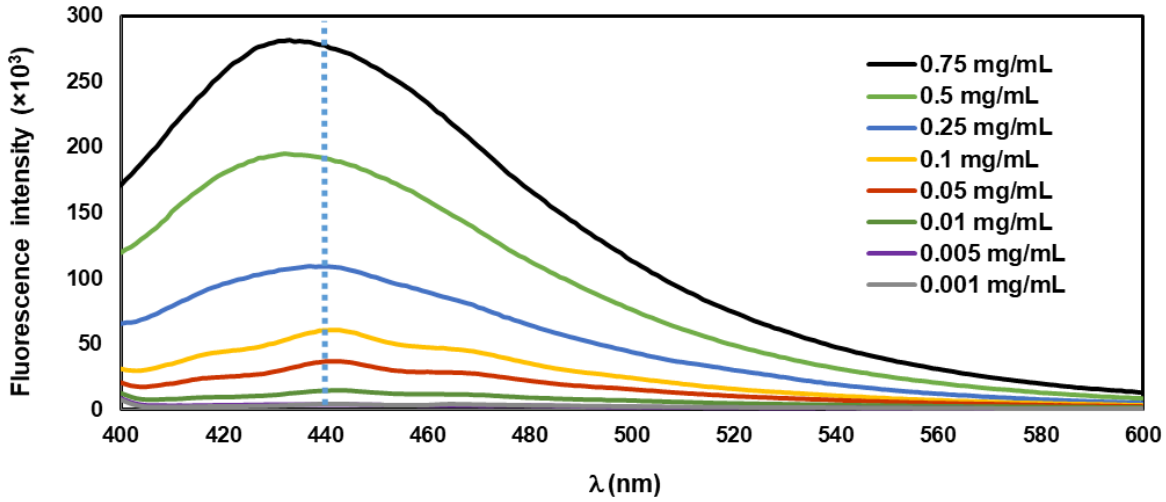

Fig. S1 Fluorescence spectra of DPH (c_{DPH} = 4 μM, λ_{ex} = 350 nm) at selected concentrations of PAMBut.

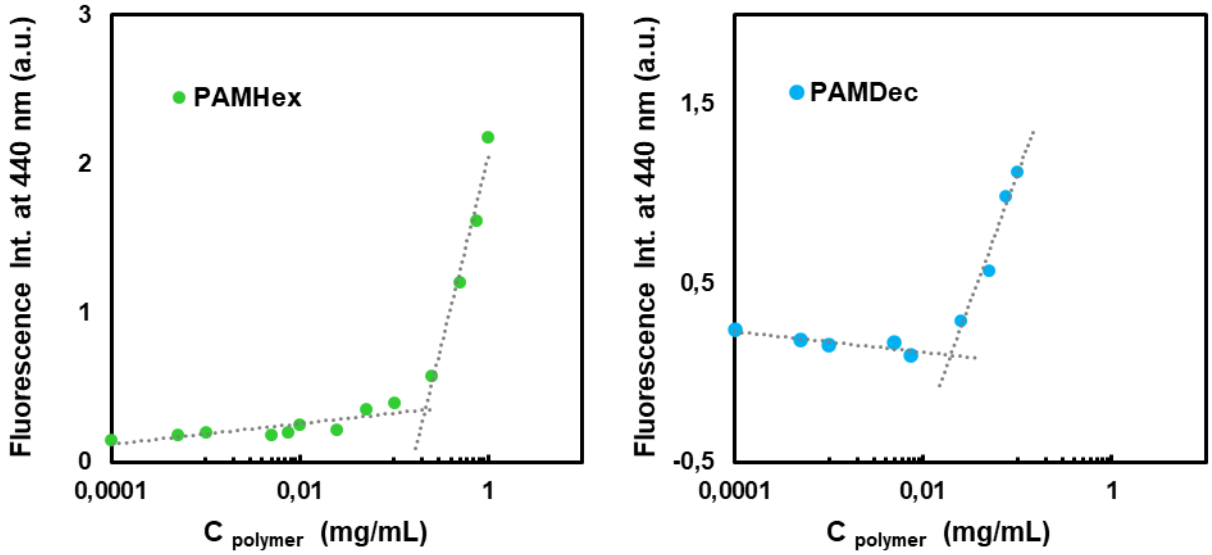

Fig. S2 The fluorescence intensity of DPH probe (cDPH = 4 μM, λex = 350 nm,) at 440 nm as a function of the concentration of PAMHex and PAMDec.

2. Dynamic light scattering (DLS) and ζ-potential measurements of polymers

The distribution profiles of the hydrodynamic diameters were measured by light scattering experiments (Zetasizer, Malvern) for the aqueous solution of PAMAlks at the concentration of 1 mg/mL. Samples were filtrated by 0.45 μm pores size filters. The mean hydrodynamic

diameter (dz), dispersity (PDI), and ζ-potential for the observed objects are presented in Table 2.

3. Dynamic light scattering (DLS) and ζ-potential - optimization of liposomes coating by polymers

Polymer-coated anionic liposomes were prepared at various lipid/polymer weight ratios in a 1 mM NaCl solution. Different volumes of the PAM and PAMAlks solutions (0.5 mg/mL) were mixed with the liposome suspension (2.5 mg in 0.5 mL) to the final volume of 1 mL. The optimal polymer content was determined from DLS and ζ-potential measurements. The crucial criteria were: small size and PDI as well as overloading the ζ-potential of the POPC/POPA liposomes to stable positive values (above 30 mV). The optimal polymer content is indicated in bold in **Table S1**.

Table S1 The values of the mean hydrodynamic diameter (dz), polydispersity index (PDI), and the ζ-potential at 298 K of the POPC/DOPA liposomes (c_{lipid} = 1.25 mg/mL) dispersed in the 1 mM NaCl solution at pH 7.4 and treated with polycations (values are the mean ± standard deviation). The chosen optimized polycation content was marked in bold.

System	Polycation content (wt%)	dz (nm) (n = 5)	PDI (n = 5)	ζ-potential (mV) (n = 8)
Liposomes (POPC/DOPA)	0	112.0 ± 0.9	0.08 ± 0.02	-42.7 ± 2.8
+ PAM	1.6	> 10^5	0.92 ± 0.18	-17.4 ± 1.4
	2.4	318.5 ± 0.3	0.34 ± 0.09	19.1 ± 1.5
	3.2	175.6 ± 0.3	0.21 ± 0.04	32.9 ± 2.6
	4.0	**153.7 ± 0.2**	**0.16 ± 0.02**	**34.2 ± 3.0**
	4.8	149.1 ± 0.2	0.21 ± 0.02	37.2 ± 2.9
	5.6	139.7 ± 0.1	0.13 ± 0.03	39.1 ± 1.1
+ PAMBut	3.2	160.2 ± 1.8	0.21 ± 0.03	19.9 ± 1.0
	4.0	153.5 ± 2.1	0.17 ± 0.02	22.4 ± 2.4
	4.8	150.1 ± 3.2	0.15 ± 0.05	27.0 ± 1.5
	5.6	**156.0 ± 3.1**	**0.13 ± 0.04**	**37.9 ± 1.8**
	6.4	147.6 ± 2.0	0.13 ± 0.03	36.0 ± 1.9
	7.2	140.5 ± 0.1	0.13 ± 0.04	36.3 ± 2.2
+ PAMHex	2.4	> 10^5	1.00 ± 0.00	-12.0 ± 1.0
	3.2	> 10^5	1.00 ± 0.00	7.0 ± 1.3
	4.0	307.6 ± 108.8	0.38 ± 0.06	20.5 ± 0.7
	4.8	146.5 ± 1.4	0.12 ± 0.02	27.0 ± 1.8
	5.6	**142.8 ± 1.2**	**0.14 ± 0.01**	**32.3 ± 2.4**
	6.4	138.0 ± 2.6	0.14 ± 0.02	33.2 ± 1.4
+ PAMOct	4.0	> 10^5	1.00 ± 0.00	0.0 ± 0.0
	4.8	> 10^5	1.00 ± 0.00	15.6 ± 0.9
	5.6	312.1 ± 10.2	0.40 ± 0.06	24.6 ± 1.4
	6.4	**209.8 ± 6.8**	**0.34 ± 0.04**	**33.3 ± 0.9**
	7.2	198.4 ± 3.8	0.30 ± 0.02	35.2 ± 1.2
	8.0	193.0 ± 2.9	0.29 ± 0.03	38.4 ± 2.4
+ PAMDec	5.6	> 10^5	1.00 ± 0.00	-7.2 ± 5.1
	6.4	> 10^5	1.00 ± 0.00	0.0 ± 0.0
	7.2	250.1 ± 10.3	0.28 ± 0.06	15.7 ± 2.6
	8.0	163.0 ± 2.4	0.14 ± 0.09	26.6 ± 3.1
	8.8	**157.5 ± 4.6**	**0.16 ± 0.08**	**31.9 ± 2.5**
	9.6	151.4 ± 1.7	0.12 ± 0.02	30.2 ± 1.0

4. Calcein release from liposomes

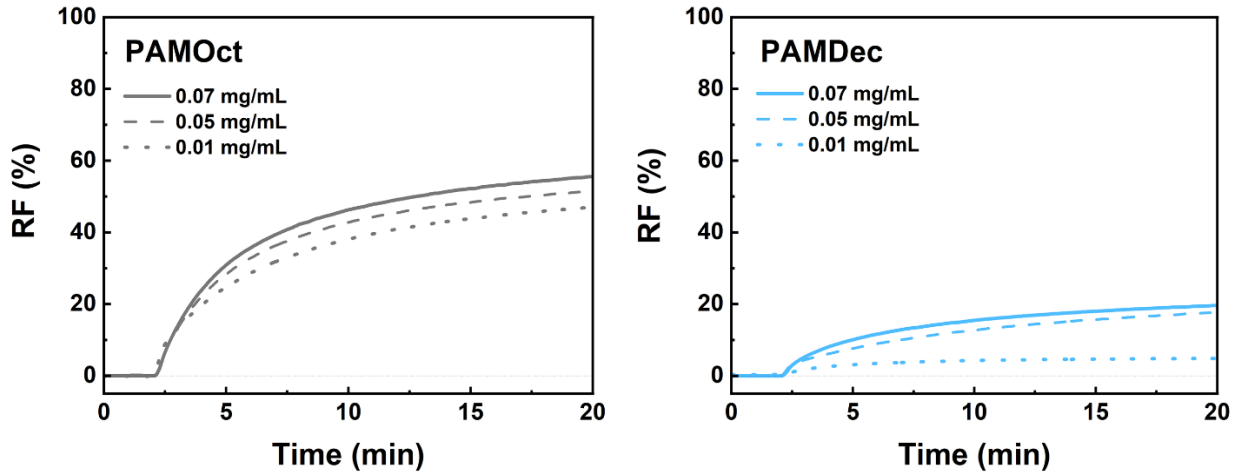

Fig. S3. Time-course of calcein leakage from the anionic SUVs treated with the polycations at 2 min. Liposomes were treated with PAMOct and PAMDec at above their CMC: 0.07 and 0.05 mg/mL and below CMC: 0.01 mg/mL. The dye was completely released by adding Triton X-100 and set as 100% leakage.

5. Calcein release from HSFs

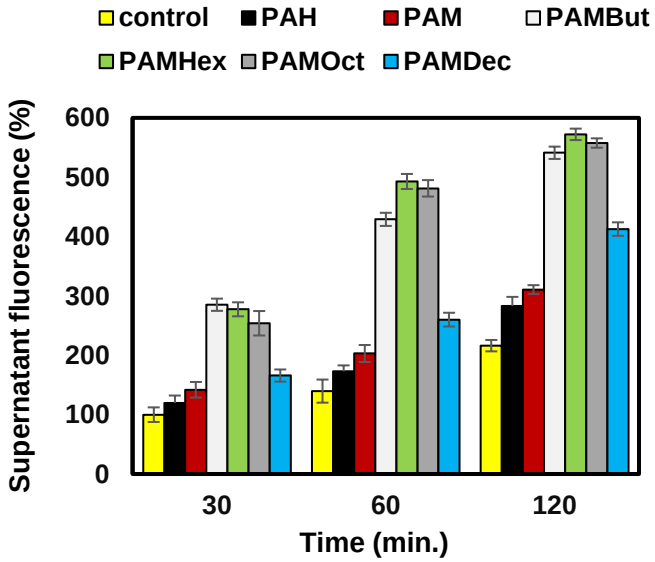

Fig. S4 Fluorescence intensity of calcein in supernatants after 30, 60, and 120 min incubation of HSFs with polymer solutions (PAH, PAM, and PAMAlks - 10 μg/mL). As a control, cells untreated with polymers were used. Average values +/- s.d. of two different experiments, each in triplicate, are shown.

6. Polymers cytotoxicity after 48h of HSFs culture.

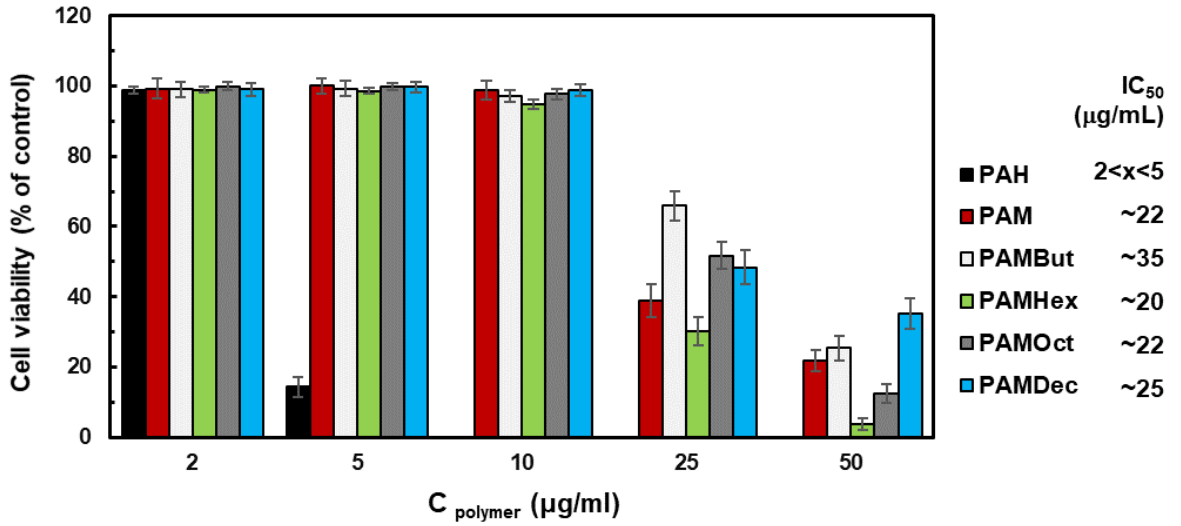

Fig. S5 Cytotoxicity of the polyelectrolytes to HSFs was measured by the FDA-EB assay. HSFs were incubated for 48 h in a medium containing the various polymers: PAH, PAM, and PAMAlks. The IC_{50} values after 48 h of incubation assessed against HSFs are given on the right.

References:

1. A. Chattopadhyay and E. London, Fluorimetric Determination of Critical Micelle Concentration Avoiding Interference from Detergent Charge, *Anal. Biochem.*, 1984, **139**, 408-412.