

Supporting Information

Title

Systematic adjustment of charge densities and size of polyglycerol amines reduces cytotoxic effects and enhances cellular uptake

Authors

Markus Hellmund,¹ Katharina Achazi,¹ Falko Neumann,¹ Bala N. S. Thota,¹ Nan Ma,^{1,2,*} and Rainer Haag^{1,*}

¹Institut für Chemie und Biochemie, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany

²Institut für Biomaterialforschung, HZG Teltow, Abteilung Biokompatibilität, Kantstr. 55, 14513 Teltow, Germany

- I. Physicochemical characterization of all hPG amines
- II. *In vitro* pretest for cell viability (all compounds)
- III. DLS measurements
- IV. Cellular uptake pictures (1 h and 4 h)
- V. Flow cytometry

I. Physicochemical characterization of all hPG amines

Sample	¹ M _w	¹ M _n	¹ PDI	² Amine loading	Amine groups	³ Zeta-potential /mV	³ Size/ d(nm)	⁴ Size/ d(nm) hPG core
PG90 14kDa	14kDa	8kDa	1.8	90%	144	18.0±0.4	7.6±1.7	5.0±0.9
PG14 60kDa	59kDa	30kDa	1.9	14%	99	18.4±0.1	16.0±0.5	7.1±1.5
PG50 60kDa	59kDa	30kDa	1.9	50%	354	20.8±1.0	18.8±4.3	7.1±1.5
PG100 60kDa	59kDa	30kDa	1.9	100%	708	15.6±0.3	15.7±0.4	7.1±1.5
PG30 100kDa	99kDa	43kDa	1.9	30%	306	23.1±0.5	7.6±3.7	7.6±0.6
PG30 200kDa	199kDa	109kDa	1.8	30%	746	23.5±4.7	12.6±5.6	9.4±0.5
PG100 200kDa	199kDa	109kDa	1.8	100%	2486	40.9±2.1	18.4±4.8	9.4±0.5
PG06 400kDa	400kDa	351kDa	1.1	6%	301	30.9±1.7	20.8±0.8	12.2±0.4
PG25 400kDa	400kDa	351kDa	1.1	25%	1252	40.6±1.6	20.5±1.3	12.2±0.4
PG86 400kDa	400kDa	351kDa	1.1	86%	4307	18.6±0.4	15.8±1.3	12.2±0.4

Table S 1. Physicochemical properties of all synthesized hPG amines. ¹M_w, M_n and PDI was determined by GPC. ²Amine loading was calculated out of the ¹H-NMR. ³DLS data measured in PBS-Buffer. ⁴DLS measurements done in Milli-Q-Water.

II. *In vitro* pretest for cell viability (all compounds)

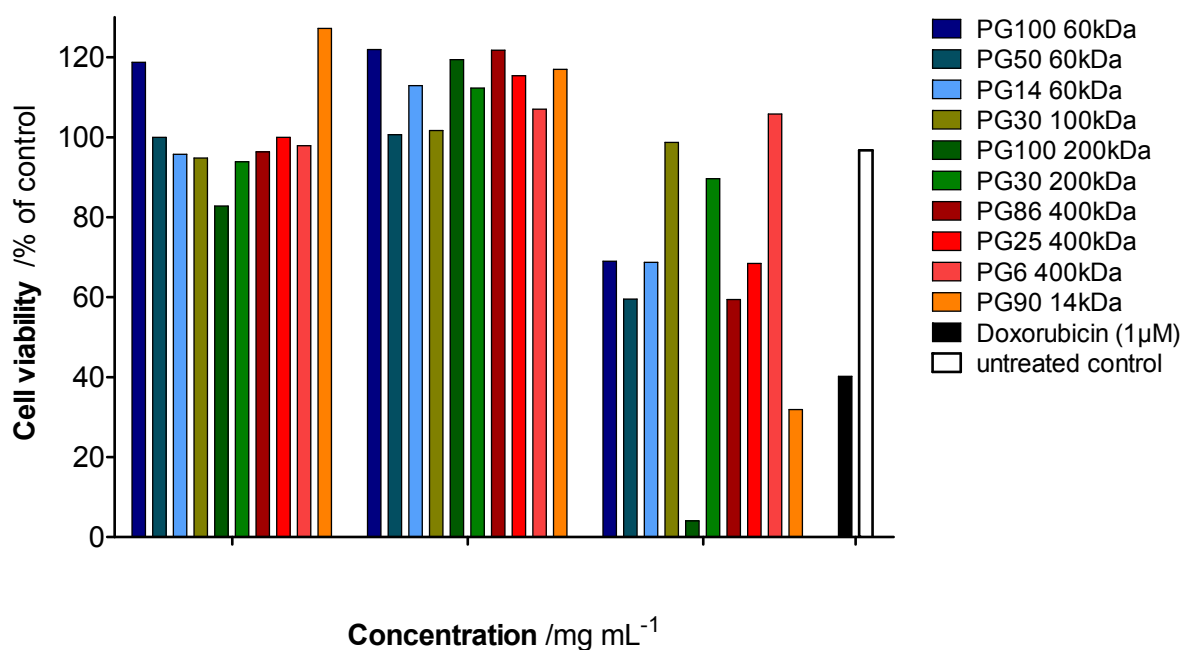


Figure S 1. *In vitro* pretest including all synthesized hPG amines. Cell viability was evaluated using A549 cells by a RTCA (real time cell analyzer) for 24 h. PG14 60 kDa, PG30 100 kDa and PG6 400 kDa are non-toxic up to 1 mg/ml (the highest concentration tested). The other hPGs were non-toxic up to a concentration of 0.1 mg/ml but showed toxicity at 1 mg/ml. The test was done in duplicates.

III. DLS measurements

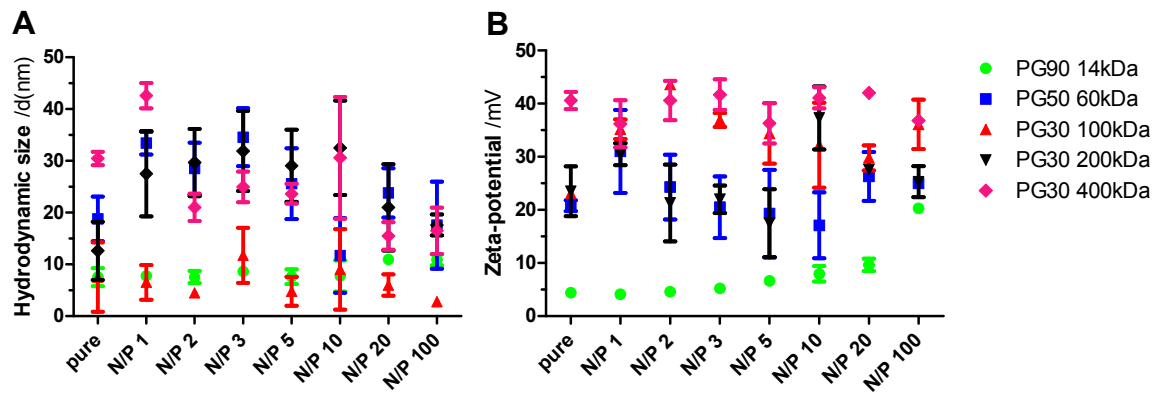


Figure S 2. DLS measurements of the compounds and polyplexes of PG90 14kDa, PG50 60kDa, PG30 100kDa, PG30 200kDa and PG25 400kDa. All measurements were performed in PBS buffer at 25°C and at an angle of 173°. Results are shown in mean±SD of at least triplicates.

IV. Cellular uptake (1 h and 4 h)

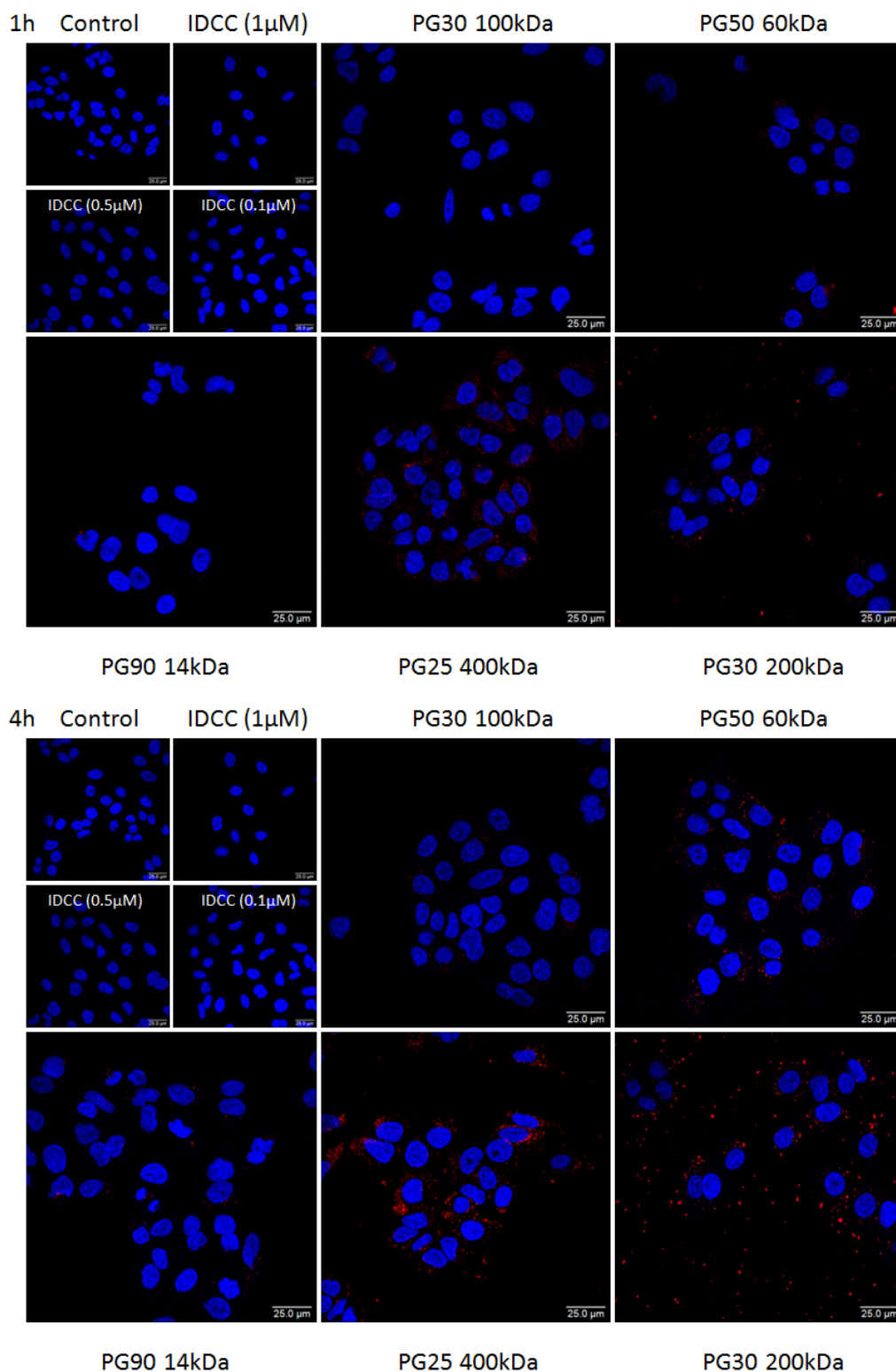


Figure S 3. Images from the cellular uptake experiments taken after 1 h and 4h incubation of A549 cells with the different IDCC-labeled nanoparticles (red). Cell nuclei were stained with DAPI (blue). Untreated cells served as control as well as three different IDCC-dye concentrations.

V. Flow cytometry

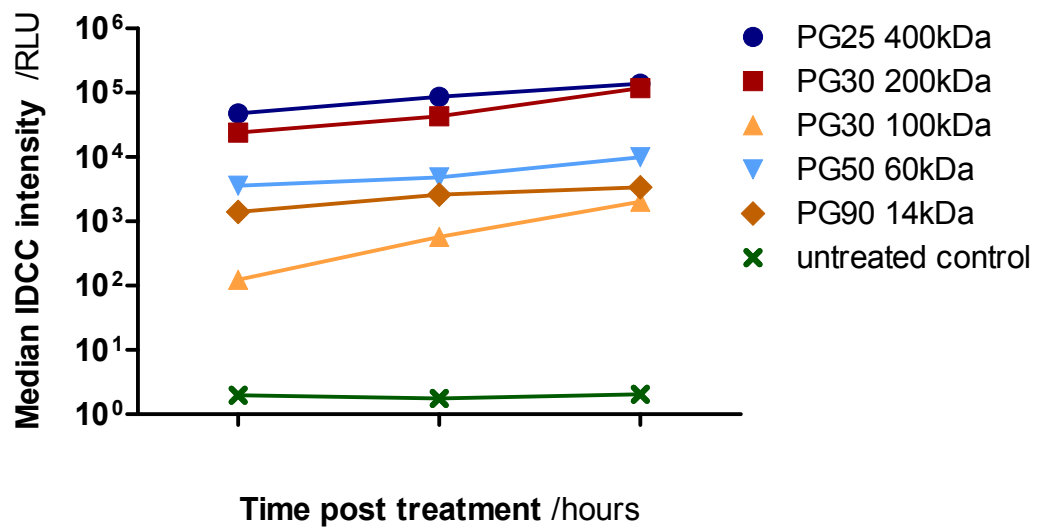


Figure S 4. Intracellular IDCC fluorescence of A549 cells 1 h, 4 h, and 24 h after treatment with the different IDCC-labeled nanocarriers measured by flow cytometry. Untreated cells served as the control. For each data point, at least 4000 single events were monitored; for the control at least 1000.