Supporting Information for:

Shell-crosslinked knedel-like nanoparticles induce lower immunotoxicity than their non-crosslinked analogs

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Table S1. The cytotoxicity (IC_{50}) of Lipofectamine, PEG-micelles and cSCKs that either PEGylated or not and with varying degrees of crosslinking. The IC_{50} values of PEGylated nanoparticles could not be determined because high cell-viabilities were observed at the range of the tested concentrations (1-100 μg/mL).

Nanoparticle	**IC_{50} (μg/mL) ± SD**	**Significance ($p < 0.05$)***
Lipofectamine	26.3±1.7	Lipofectamine has significantly lower IC_{50} than the cSCKs
5%-cSCKs (5% crosslinking)	46.6±5.4	
PEG-micelles (0% crosslinking)	-----	
PEG-5%-cSCKs (5% crosslinking)	-----	
PEG-20%-cSCKs (20% crosslinking)	-----	

*Values are presented as mean ± SD of at least three independent experiments. Significant differences between two groups were evaluated by Student's t test (unpaired) or between more than two groups by one-way ANOVA followed by Tukey's multiple comparison test. Differences between different groups were considered significant for p values < 0.05.

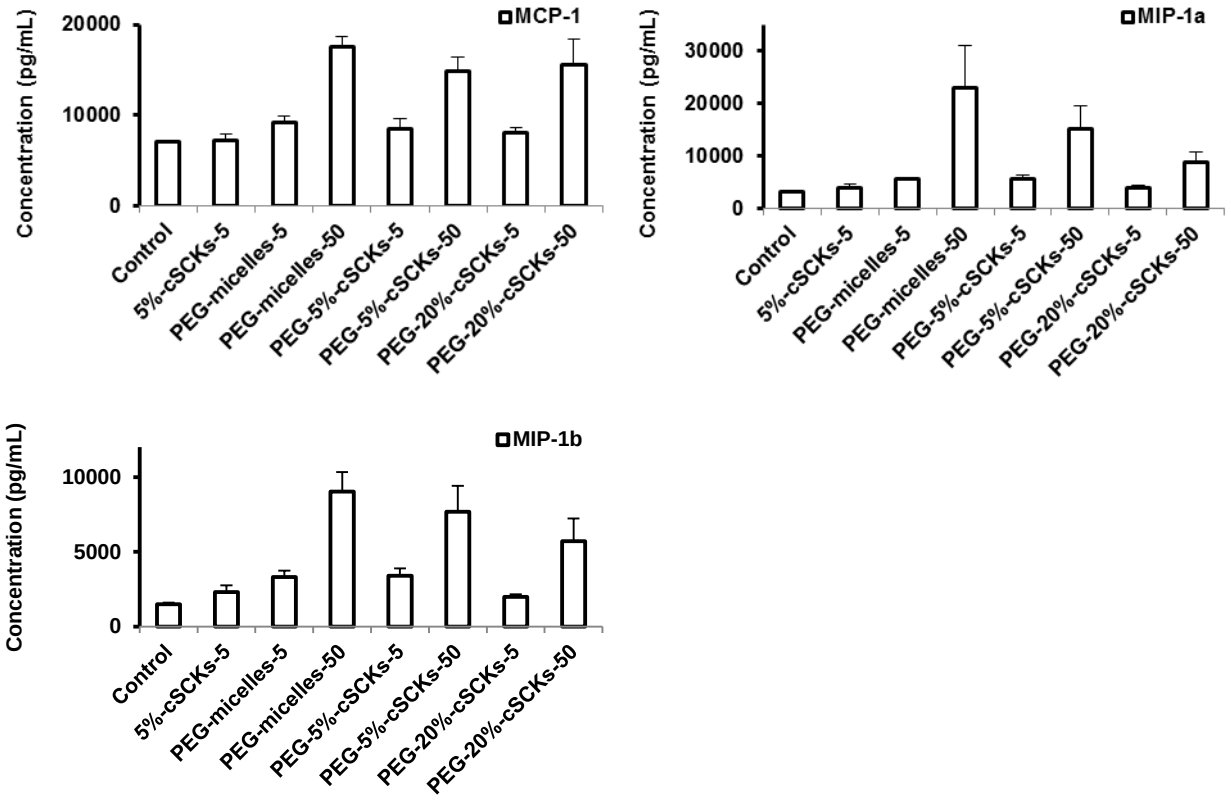

Figure S1. Cytokine induction data for control, 5%-cSCKs (5 μg/mL), PEG-micelles (5 and 50 μg/mL), PEG-5%-cSCKs (5 and 50 μg/mL) and PEG-20%-cSCKs (5 and 50 μg/mL). The expression of MCP-1, MIP-1α and MIP-1β was particularly enhanced upon the PEGylation of micelles and 5%-cSCKs even for the 20%-crosslinked PEG-cSCKs.

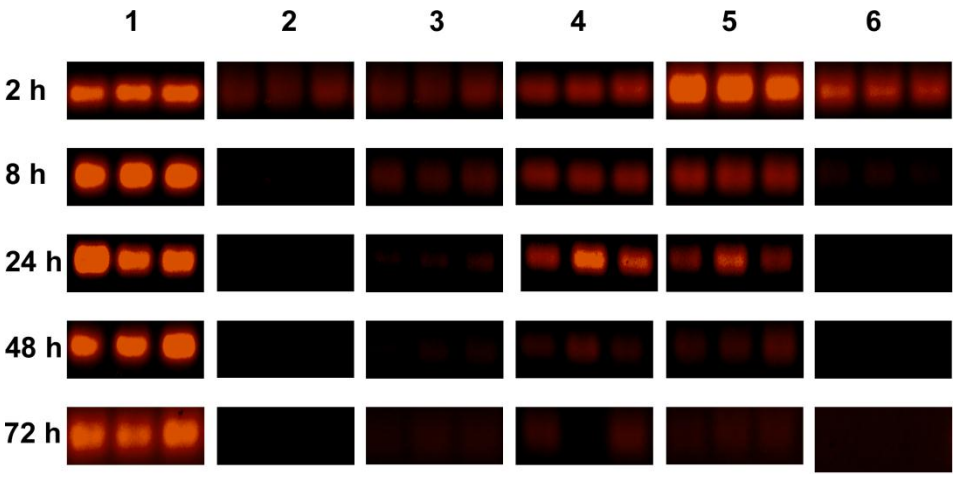

Figure S2. Degradation of Cy3-labeled siRNA (0.6 µg siRNA/42.7 µL, 10 mM Tris, pH 7.4) that was incubated in 87% fetal bovine serum after 2, 8, 24, 48 and 72 h: (1) siRNA in water, (2) siRNA in serum, siRNA complexed with (3) 5%-cSCKs, (4) PEG-micelles, (5) PEG-5%-cSCKs and (6) PEG-20%-cSCKs.

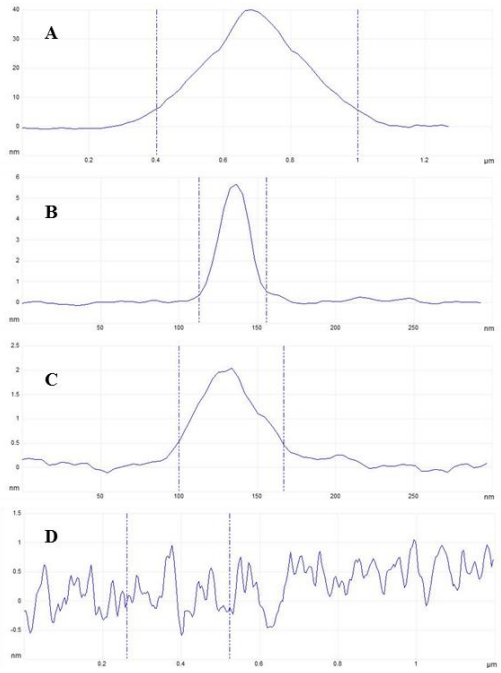

Figure S3. Typical AFM topography profiles of the PEG-20%-cSCKs (A), PEG-5%-cSCKs (B), PEGylated micelles (C) and non-PEGylated 5%-cSCKs (D) after deposition on mica.

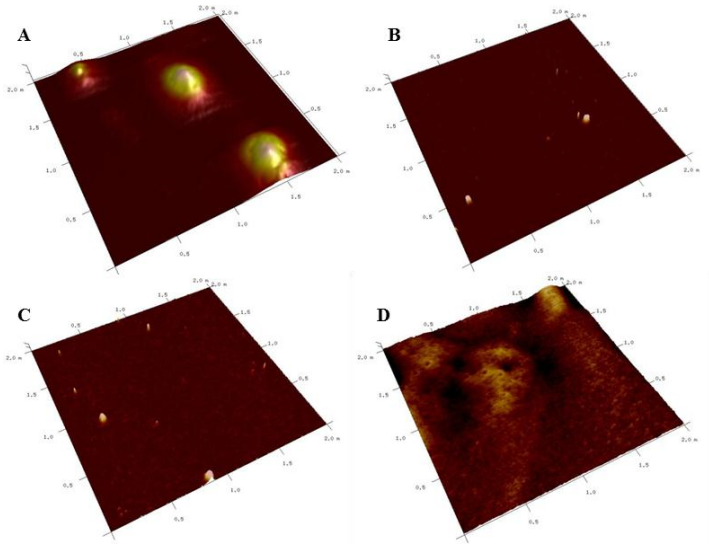

Figure S4. 3D Renderings of AFM topography for the PEG-20%-cSCKs (A), PEG-5%-cSCKs (B), PEGylated micelles (C) and non-PEGylated 5%-cSCKs (D) after deposition on mica.