Supporting Information

Nioplexes Encapsulated in Supramolecular Hybrid Biohydrogels as Versatile Delivery Platforms for Nucleic Acids

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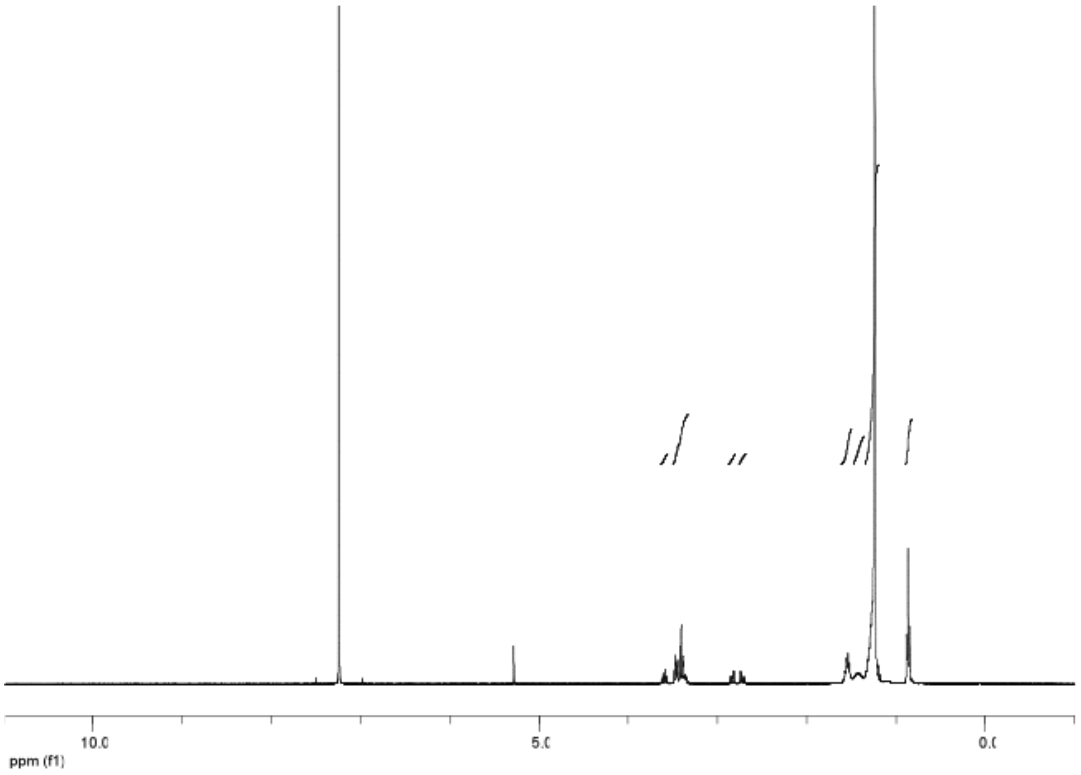

Figure S1. ^{1}H-NMR of *lipid*-**1**. Reaction was performed under argon atmosphere. ^{1}H-NMR spectra was recorded in $CDCl_3$ at 25 °C on a Varian Mercury 400 MHz spectrometer. The proton signal for residual non-deuterated solvent (δ 7.26) was used as an internal referente. Chemical shifts are reported in parts per million (ppm), coupling constants (*J*) in Hz and multiplicity as follows: t (triplet), dd (doublet of doublets), m (multiplet): ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 3.59 (m, 1H; C<u>H</u>-O), 3.46 (m, 2H; C<u>H</u>$_2$-O), 3.40 (m, 4H; 2 C<u>H</u>$_2$-O), 2.83 (dd, *J* = 13.4 Hz, 3.9 Hz; 1H; C<u>H</u>-N), 2.71 (dd, *J* = 13.2 Hz, 4.0 Hz; 1H; C<u>H</u>-N), 1.53 (m, 4H; 2 C<u>H</u>$_2$-C), 1.23 (m, 40 H; alkyl chain), 0.86 (t, *J* = 7.0 Hz, 6H; 2 C<u>H</u>$_3$-CH$_2$).

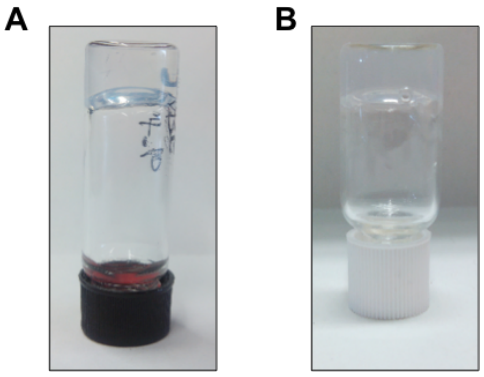

Figure S2. Supramolecular hydrogel picture selections. (A) *N*-Fmoc-protected amino acid (Fmoc-Phe-OH) containing cationic niosomes (*hydrogel*-**1**). (B) *N*-Fmoc-protected amino acid (Fmoc-Phe-OH) crosslinked with κ-carrageenan (1%, w/v) (*hydrogel*-**4**).

Table S1 Encapsulation efficiencies (EE) of *hydrogels*-(**1**-**4**). Native hydrogels (*hydrogel*-**5** and *hydrogel*-**6**) were used as controls for comparison purposes.[a]

Entry	Sample	κ-C (%, w/v)	Cationic niosomes: FITC-ODN	EE (%)	G' (kPa)	G'' (kPa)	γ (%)	γ_c (%)	tan δ
1	*Hydrogel*-**1**	-	yes	94.0±1.5	2.68	0.40	-	16.6	0.15
2	*Hydrogel*-**2**	0.5	yes	97.0±0.5	nd	nd	nd	nd	nd
3	*Hydrogel*-**3**	0.8	yes	98.0±0.4	nd	nd	nd	nd	nd
4	*Hydrogel*-**4**	1	yes	98.0±0.8	5.29	0.57	87	20.0	0.10
5	*Hydrogel*-**5**	-	native	-	2.38	0.33	43	13.0	0.14
6	*Hydrogel*-**6**	1	native	-	7.81	0.72	68	13.4	0.09

[a] Abbreviations and definitions: κ-C = κ-carrageenan; G' = storage modulus; G'' = loss modulus; γ = strain at break (yield stress); γ_c = critical strain; tan δ = loss factor (G''/G'); nd = not determined. Reported data are means of three independent experiments ± S.D.

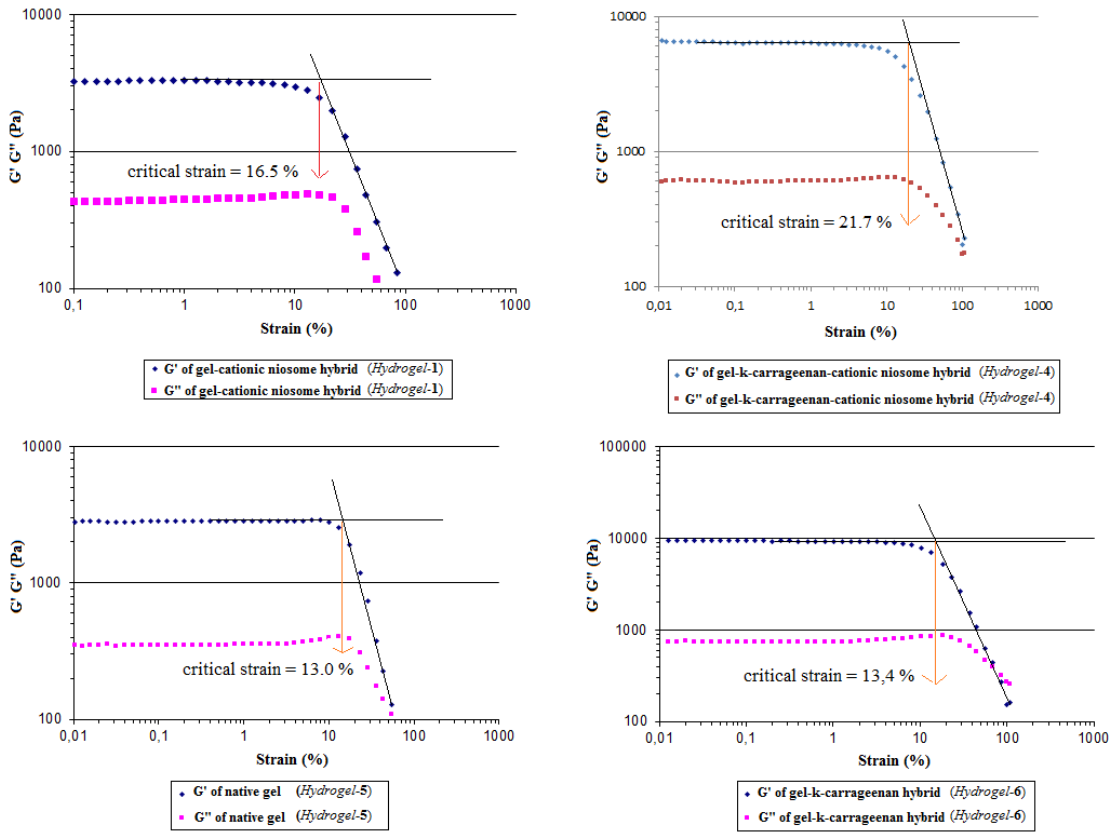

Figure S3. DSS measurements and critical strain (γ_c).

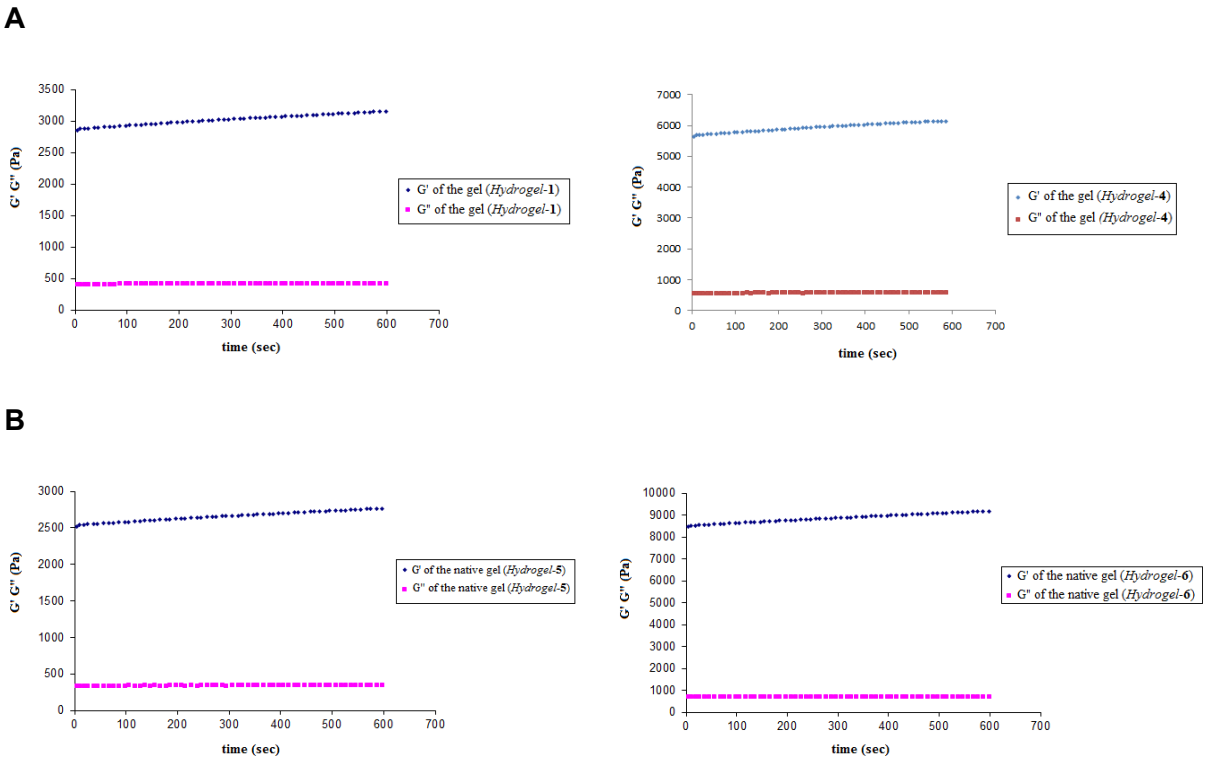

Figure S4. DTS measurements of *hydrogel*-**1**, *hydrogel*-**4**, *hydrogel*-**5** and *hydrogel*-**6**. (A) *Left*: Hydrogel containing cationic niosomes (*hydrogel*-**1**). *Right*: hydrogel crosslinked with κ-carrageenan (1%, w/v) and containing cationic niosomes (*hydrogel*-**4**). (B) *Left*: Native hydrogel (without cationic niosomes and κ-carrageenan; *hydrogel*-**5**). *Right*: Native hydrogel (without cationic niosomes but containing κ-carrageenan; *hydrogel*-**6**).

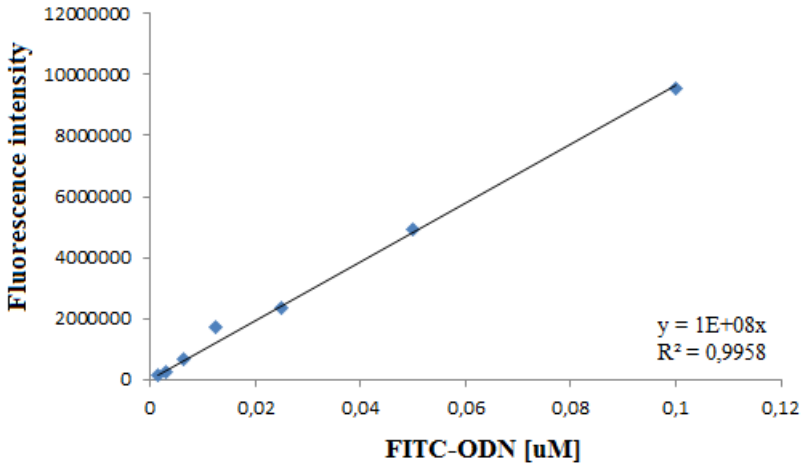

Figure S5. Standard curve of FITC-ODN.

Table S2. Model release parameters for *hydrogels*-(**1**-**4**) according to Higuchi equation

$$\frac{M_t}{M_\infty} = k * \sqrt{t}$$

Entry	Sample	*k*	r^2
1	*Hydrogel*-**1**	9.74	0.9891
2	*Hydrogel*-**2**	6.90	0.9717
3	*Hydrogel*-**3**	5.73	0.9765
4	*Hydrogel*-**4**	5.12	0.9947

Table S3. Model release parameters for *hydrogels*-(**1**-**4**) according to Korsmeyer-Peppas' equation. The model was calculated for the first 60% of the FITC-ODN release

$$\frac{M_t}{M_\infty} = k * t^n$$

Entry	Sample	*k*	*n*	r^2
1	*Hydrogel*-**1**	7.63	0.56	0.9935
2	*Hydrogel*-**2**	6.04	0.52	0.9940
3	*Hydrogel*-**3**	5.38	0.51	0.9938
4	*Hydrogel*-**4**	5.12	0.52	0.9952

Table S4. Model release parameters for *hydrogels*-(**1**-**4**) according to Weibull equation

$$\frac{M_t}{M_\infty} = a * (1 - \exp(-(kt)^b))$$

Entry	Sample	*a*	*k*	*b*	r^2
1	*Hydrogel*-**1**	137.8	0.013	0.77	0.9803
2	*Hydrogel*-**2**	98.9	0.013	0.96	0.9647
3	*Hydrogel*-**3**	94.5	0.011	0.93	0.9834
4	*Hydrogel*-**4**	129.7	0.005	0.73	0.9949

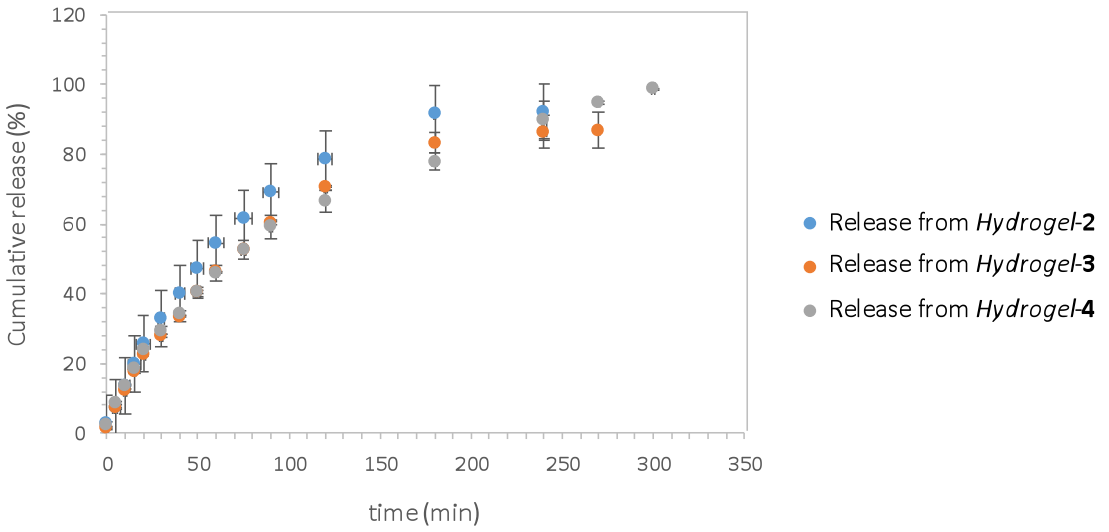

Figure S6. Combination of the three niosomal FITC-ODN cumulative release from *hydrogels*-(**2**-**4**).

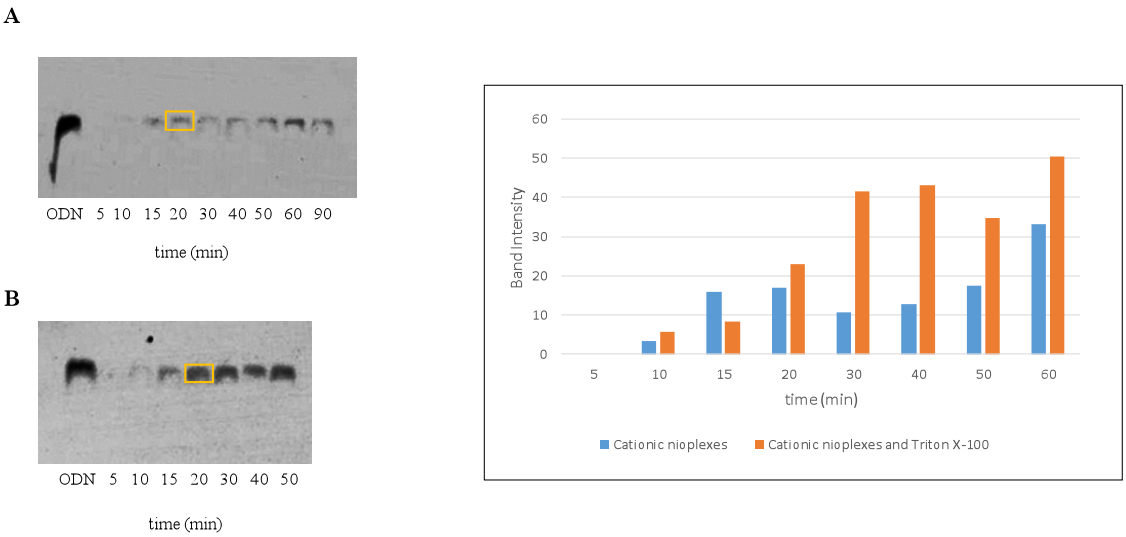

Figure S7. A. FITC-ODN release from *hydrogel*-**3** containing cationic nioplexes analyzed by native gel polyacrylamide electrophoresis (PAGE). B. FITC-ODN release from *hydrogel*-**3** containing cationic nioplexes analyzed by native gel polyacrylamide electrophoresis. 100 mM Triton X-100 was added to the PBS receptor phase at different times. The solubilization of the niosomes produced a liberation of the unformulated FITC-ODN.

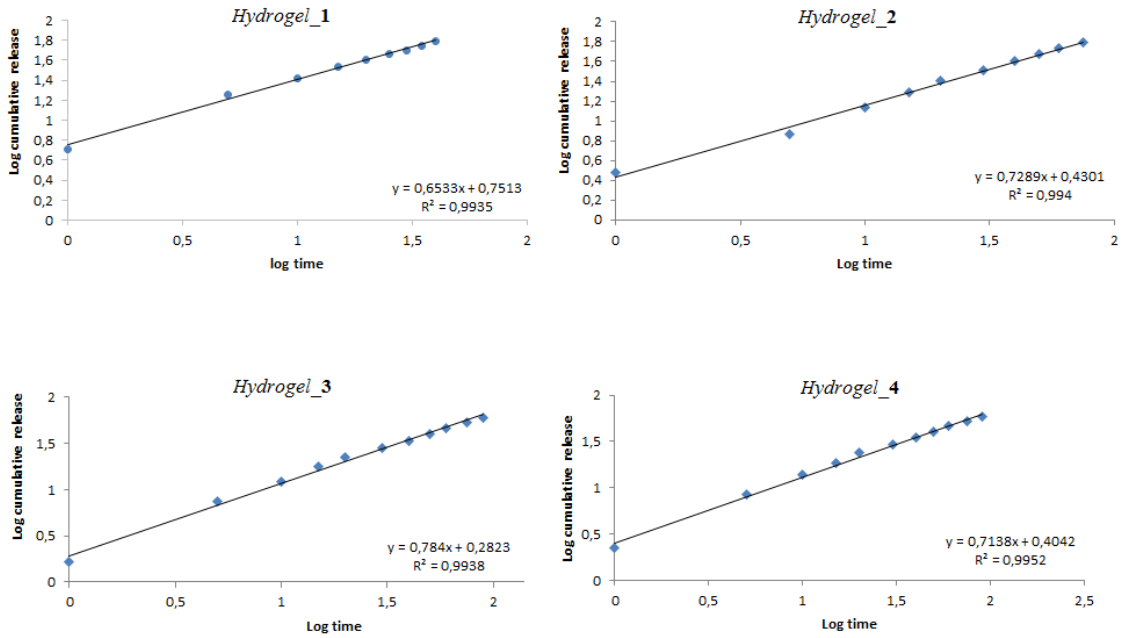

Figure S8. Release profiles of niosomal FITC-ODN from *hydrogels*-(**1**-**4**).

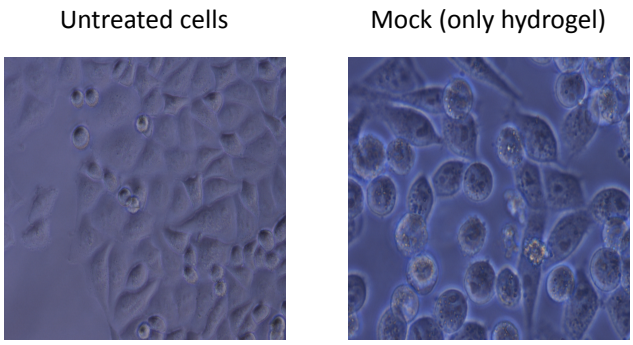

Figure S9. Cell morphology images of HeLa cells in the absence (*left*) and the presence of *hydrogel*-**3** (*mock*) (*right*).