Supplementary Information

High Efficiency and Low Toxicity of Polyethyleneimine Modified Pluronics (PEI-Pluronic) as Gene Delivery Carriers in Cell Culture and Dystrophic *mdx* Mice

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1. Characterization of polymers:

The Pluronics were first activated by CDI followed by reaction with excess of the low molecular weight PEI (0.8k, 1.2k). This procedure should make the LPEI conjugate to the terminal ends of pluronics.^{29,34} it's also confirmed by ¹H NMR (Fig. S1) and MALDI-TOF-MASS (PCM-05 in Fig. S2). The conjugate PEI % of polymers were further determined by nitrogen element analysis, the results showed that the Pluronics had very high conjugating percent with PEI from 77.5% to 95.4% (Table S1).

	Mw of reactants							Percentage of
Code			PEI	Mw of	PEI % of	N /%	N /%	Pluronic
	Dhunonio			PCMc	PCMe	(Cole.)*	(Found	modification
	Pluronic			1 CIVIS	I CIVIS	(Calc.)	(Found	(%)
	Mw(Da) ^a	HLB ^{b,}		(Da,	(Calc.))	
				Calc.)				
PCM-01	L64	12-18	800	4500	35.6	11.85	10.94	92.3
	(2900)							
PCM-02	P85	12-18	800	6200	25.8	8.60	7.60	88.4
	(4600)							
PCM-03	F127(126	18-23	800	14200	11.3	3.75	2.99	79.7
	00)							

Table S1. Characteristics of PCM polymers)

PCM-04	L64	12-18	1200	5300	45.3	15.09	13.08	86.7
	(2900)							
PCM-05	P85	12-18	1200	7000	34.3	11.43	9.76	85.4
	(4600)							
PCM-06	F127(126	18-23	1200	15000	16.0	5.33	4.32	81.2
	00)							
PCM-07	L35	18-23	800	3500	45.7	15.23	13.83	90.8
	(1900)							
PCM-08	L44	12-18	800	3800	42.1	14.03	12.33	87.9
	(2200)							
PCM-09	L35	18-23	1200	4300	55.8	18.60	15.75	84.7
	(1900)							
PCM-10	L44	12-18	1200	4600	52.2	17.39	14.35	82.5
	(2200)							
PCM-11	P123	7-12	800	7350	21.8	7.26	5.62	77.5
	(5750)							
PCM-12	P123	7-12	1200	8150	29.5	9.82	7.86	80.2
	(5750)							
PCM-13	PEG-	hydroph	800	7600	21.1	7.02	6.70	95.4
	6000 ^e	ilic						
PCM-14	PEG-	hydroph	1200	8400	28.6	9.52	8.79	92.3
	6000 ^e	ilic						

• Nitrogen content was calculated theoretically as 33 wt% in PEI.

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Fig. S1. Representative ¹H-NMR Spectra of Pluronic-PEI in D₂O (JEOL 500).



Fig. S2. MALDI-TOF-MASS of PCM -05 (Sinapinic acid with 0.1% TFA)

MALDI-TOF Mass values of polymer was determined using a Voyager-DE Pro STR MALDI-TOF mass spectrometer. Samples were prepared by (1) plate coating with 0.5 μ L saturated sinapinic acid in 50% acetonitrile with 0.1% TFA; (2) depositing 0.5 μ L solution of polymer in water (10⁻⁵ M); and, finally, (3) coating with 0.5 μ L saturated sinapinic acid in 20% acetonitrile with 0.1% TFA.

2. DNA binding comparison for Pluronic only and Pluronic-PEI conjugate: The Pluronic-PEI conjugates (PCMs) showed strong bind with negatively charged DNA, but the with pluronic (such as P85) showed same as DNA only(Fig. S3).



Fig. S3. Electrophoretic mobility of DNA mixed with polymers (The complexes were prepared immediately before use by gently vortexing a mixture of DNA and polymer solution at weight ratio of polymer/DNA =5. The complexes were incubated at room temperature for 30 minutes in 24 μ L volume, then loaded onto 1% agarose gel with ethidium bromide (0.1 μ g/mL) in tris-acetate (TAE) buffer (100V, 40min). The gel was analyzed on UV illuminator).