Supplementary Information

Sperm-like Nanocarriers for Ultrafast Delivery of Antisense DNA

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Experimental Section

Materials and reagents

All DNA oligonucleotides (sequences were shown in Table S1) were synthesized by Sangon Biotechnology Inc. (Shanghai, China) and purified by high-performance liquid chromatography (HPLC). Hoechst 33342, Cell Counting Kit-8 (CCK-8) and Annexin V-FITC Reagent were purchased from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China). Methyl-beta-cyclodextrin (MβCD), chlorpromazine (CPM), amiloride (AMI), nystatin (NYS), and dynasore (DYN) were acquired from Sigma Aldrich (St. Louis, MO, USA). The primary antibodies of GAPDH and Survivin, and the secondary antibody of rabbit IgG (HRP-labeled) were purchased from Abcam Co., Ltd. (Cambridge, MA, USA). ECL Western Blotting Substrate was purchased from Solarbio Science and Technology Co. Ltd. (Beijing, China). Exonuclease I and Exonuclease III were purchased from Thermo Fisher Scientific Co., Ltd. (Waltham, MA, USA). All used chemical reagents were analytical grade without further purification. CH_3COONH_4 (NH_4Ac), $AgNO_3$, $NaBH_4$, and other chemicals were acquired from Sigma Aldrich (St. Louis, MO, USA). All solutions were prepared with Milli-Q water (18.2 $M\Omega \cdot cm^{-1}$) from a Milli-Q purification system (Millipore, Milford, MA, USA).

Synthesis of AgNCs and nano-sperms

DNA stabilized AgNCs were prepared following literature method.[1] DNA template (2.5 μM) was added to the mixture contained NH_4Ac (10 nM) and $AgNO_3$ (50 μM). After vortexed mixing for 1 min, the solution was kept in dark at 4 °C for 2 h for further experiments.For synthesis of nano-sperms, four different DNA as the “tail” at the 5' end of nano-sperms (including ssDNA, dsDNA, hairpin DNA, and quadruplex DNA) were first denatured at 95 °C for 5 min and then cooled to room temperature. AgNCs were synthesized using the DNA template as the “head” at 3' end of nano-sperms. After the reaction was completed, the nano-sperms were stored at 4 °C for use on the day.

Characterization of AgNCs and nano-sperms

The UV-vis spectroscopy was applied for characterization of the absorbance of AgNCs using a spectrophotometer (Shimadzu UV-2450, Shimadzu Corporation, Japan). The fluorescence intensity was obtained by F-7000 spectrofluorometer (Hitachi High-Technologies, Japan) with an excitation wavelength of 574 nm and an emission wavelength of 650 nm. For transmission

electron microscopy (TEM) and high-resolution TEM, the AgNCs were characterized using JEM-2100 transmission electron microscopy (JEOL, Japan). For atomic force microscope (AFM) characterization, AgNCs and nano-sperms were deposited on the surface of mica substrates (Yunfeng Co. Ltd., China) and characterized using Agilent 5500 atomic force microscope system (Santa Clara, CA). The size distribution and potential of AgNCs and nano-sperm (ssDNA) were measured by ZETASIZER 3000HS instrument (Malvern Instruments Ltd., UK).

Gel electrophoresis analysis

The reaction products of nano-sperms with different DNA structures were analyzed by polyacrylamide gel electrophoresis (PAGE). 10 μl of each sample with 2 μL of 6 × loading buffer and 1μL of SYBR Green I was loaded onto 15% non-denaturing polyacrylamide gel, and electrophoresed in 1 × Tris boric acid EDTA (TBE) at 120 V for 90 min. The imaging of the gel was performed using Gel Doc XR Imaging System (Bio-Rad, USA).

To study the antihydrolysis of nano-sperm (ssDNA), samples were treated with 10 U Exonuclease I and 50 U Exonuclease III at 37 °C for 30 min, then analyzed by PAGE. To study the stability, samples were incubated with DMEM containing 10% FBS at 37 °C for 0, 0.5, 1, 2, 4, 6, 12, or 24 h, then analyzed by PAGE. The ssDNA was used as control.

Cell culture

HeLa cells (human cervical cancer cell) were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). HeLa cells were cultured in Dulbecco modified Eagle medium (DMEM, Gibco, Invitrogen) with 10% fetal bovine serum (FBS, Gibco, Invitrogen) and 1% antibiotics (penicillin-streptomycin-neomycin) in a humidified atmosphere at 37 °C containing 5% CO_2. Cells were selected at the end of the log phase.

Delivery of nano-sperms into cells

1×10^4 HeLa cells were seeded into a 15 mm confocal dish in folate-free DMEM and cultured at 37 °C with 5% CO_2 for 24 h. After 24 h, HeLa cells were washed with phosphate-buffered saline (PBS, pH 7.4) for three times. Then cells were incubated with medium contained 1 μM DNA or nano-sperms in the dark to for different time. After washing, cells were imaged with LSM 710 Zeiss confocal microscope (Zeiss, Germany). The FAM labeled DNA was imaged

using a green filter, and the AgNCs was imaged using a red filter. Fluorescence intensity was analyzed using Image J software.

For comparison with liposomes, Lipofectamine 2000 transfection reagent (Life Technologies, Grand Island, NY) and 1 μM ssDNA were electrostatically complexed by mixing them in a molar ratio of 5:1. Cells were treated with nano-sperms and liposomes at 37 °C for 1 h or 4 h, respectively.

In order to study the cellular uptake mechanism of nano-sperms, cells were pretreated with 10 mM MβCD, 100 μM CPM, 100 μM AMI, 4 μM NYS or 4 μM DYN respectively for 1 h at 37 °C. After washing with PBS for three times, nano-sprems were added and incubated for another 1 h. After washing, cells were imaged with LSM 710.

Cytotoxicity of nano-sperms

For cell apoptosis assays, 1×10^5 HeLa cells were seeded into a 6-well plate and cultured for 24 h. After washing, cells were treated with 1 μM AgNCs or 1 μM nano-sperms carried different "cargo" for 1 h. The group with only cells was used as the control. Afterwards, cells were collected and stained with Annexin-V FITC for 15 min, then analyzed using CytoFLEX Flow Cytometer (Beckman Coulter, USA).

For cell viability assays, 1×10^4 HeLa cells were seeded and cultured for 24 h in 96-well plates. After washing, cells were treated with the above groups for 1 h. The cells were then incubated with 10 μL of CCK-8 solution and 100 μL of DMEM at 37 °C for 2 h. Absorbance was obtained using a microplate reader at a wavelength of 450 nm using SpectraMax M2 Multi-Mode Microplate Reader (Molecular Devices, USA).

Western blot analysis

HeLa cells were seeded into a 15 mm confocal dish for 24 h, and then were treated with 1 μM AgNCs or 1 μM nano-sperms (asDNA) for 4 h when it became 80% full. The group with only cells was used as the control. After washing with PBS, cells were cultured for another 48 h. The total proteins were extracted from the treated cells using RIPA lysis buffer (KeyGEN, Nanjing, China) containing PMSF on ice. 25 μg proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to pure nitrocellulose blotting membranes (Millipore, Milford, MA, USA). The membranes were blocked with 2% BSA at room temperature for 2 h, followed by incubation with primary antibodies overnight at 4 °C. After washing, the membranes were incubated with secondary antibodies at room temperature for 2 h. Finally, the membranes were visualized using a ChemiDocTM XRS Plus luminescent

image analyzer (Bio-Rad, USA). Image J software was used to analysis relative protein expression by calculating the gray-scale blots.

Statistical Analysis

All data were expressed as the mean ± standard error. One-way analysis of variance (ANOVA) by GraphPad Prism (GraphPad Software, La Jolla, CA) was used to compare the differences. Difference with $p < 0.05$ was considered to statistically significant.

References

[1] G. Jie, L. Tan, Y. Zhao, X. Wang, *Biosens. Bioelectron* **2017**, *94*, 243-249.

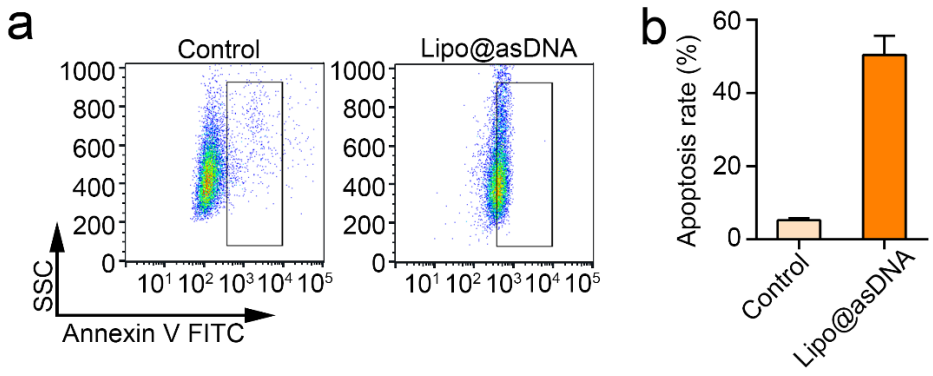


Fig. S1 Cell apoptosis analysis of flow cytometry assays in HeLa cells transfected with liposome-encapsulated asDNA (Lipo@asDNA). The means from three biological replicates (n = 3) are displayed.

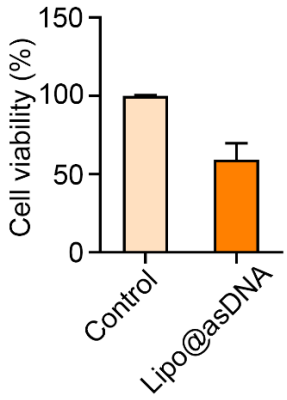


Fig. S2 Cell viability quantification of CCK-8 assay in HeLa cells transfected with liposome-encapsulated asDNA (Lipo@asDNA). The means from three biological replicates (n = 3) are displayed.

Table S1 Sequences of oligonucleotides.

Oligonucleotides	Sequence (5′-3′)
DNA template	CCCCCCCCCCCCCCC
Nano-sperm (ssDNA60)	GCTCTACCGATAGCCGCTAGGTTAGTACATCTTACAGCATT TCTTCCCCCCCCCCCCCCC
Nano-sperm (ssDNA)	FAM-ACTCCATCTGGTATTACTACTCCCCCCCCCCCCCCC
Nano-sperm (dsDNA-1)	CCCAGCCTTCCAGCTCCTTGACCCCCCCCCCCCCCC
dsDNA-2	TCAAGGAGCTGGAAGGCTGGG-FAM
Nano-sperm (Hairpin)	FAM-GTCGAGTTTTGTTGTTGGTCTCGACCCCCCCCCCCCCCCC
Nano-sperm (Quadruplex DNA)	FAM-GGTGGTGGTGGTTGTGGTGGTGGTGGCCCCCCCCCCCCCCC
Nano-sperm (asDNA)	CCCAGCCTTCCAGCTCCTTGACCCCCCCCCCCCCCC

Figure 2a

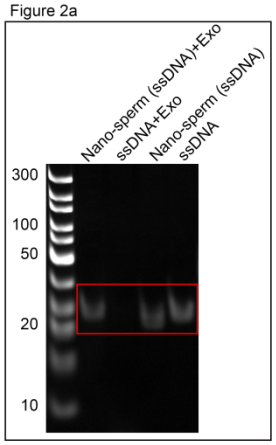


Figure 2b

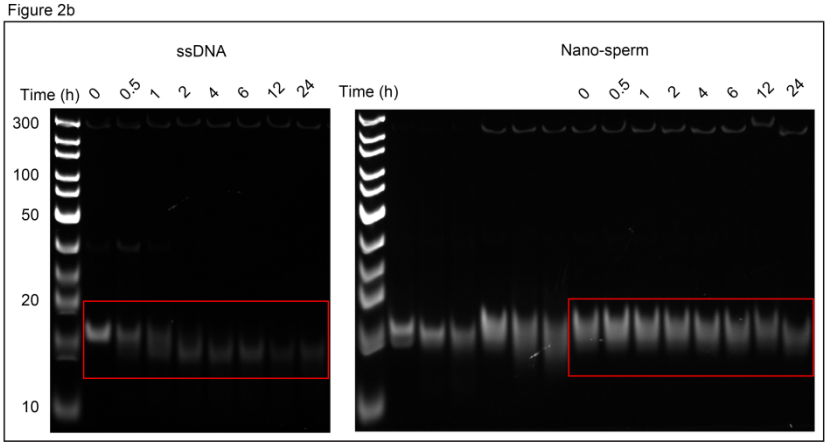
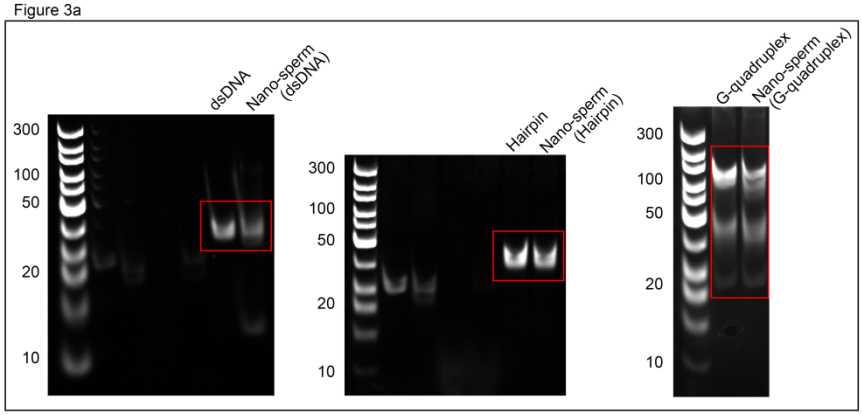


Figure 3a



Raw data