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BN nanospheres as CpG ODN carrier for activation of toll-like receptor 9

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Supporting Information

1. Materials and Methods

Preparation of BN nanospheres

A traditional tube style CVD system was used to synthesize BN nanospheres. $B(OMe)_3$ and NH_3 were loaded into the furnace. The exhausting gas that contained ammonia was fed through a water or acid solution bath to collect the remnant toxic

gas. Thus, the CVD system was kept under a positive pressure. Growth temperature was kept at 950 °C. A product was collected from the downstream side slightly away from the furnace center. The collected product was further annealed under protection of ammonia at 1375 ℃.

DNA sodium salt loading and releasing of BN nanospheres

For loading DNA sodium salt on BN nanospheres, 200 mg DNA sodium salt (Deoxyribonucleic Acid sodium salt from salmon spermary produced by Wako company) was added to pure water and the solution was heated up to 90℃ and stirred for 1h. Then 500 mg pristine BN nanospheres were mixed, followed by 24h stirring. Finally, BN nanospheres were centrifuged out, washed and dried.

For releasing, a 20 mg DNA sodium salt loaded BN nanospheres hybrid was added to phosphate-buffered saline solution (PBS, 20 ml, PH 5.0), and stirred at 150 rpm at room temperature. At certain time intervals, aliquots of PBS (4 ml) were taken out by centrifugation to test the concentration of released DNA sodium salt, and 4 ml fresh PBS was added to the solution of DNA sodium salt loaded BNNSs.

Cell cultures

293XL-hTLR9 cells stably expressing human TLR9 were purchased from “Invivogen” Company (California, US). Cells were grown in DMEM medium supplemented with 10 (v/v) % FBS, 50 units/ml penicillin, 50 mg/ml streptomycin, 100 μg/mL normocin and 10 μg/mL blasticidin at 37 °C in humidified air containing

5% CO_2. Cells were seeded on 24-well culture plates for transfection and stimulation experiments.

Unmethylated cytosine-phosphate-guanine (CpG) oligodeoxynucleotide(ODN)

3'-Fluorescein isothiocyanate (FITC) labeled natural phosphorodiester CpG ODN 2006 was purchased from "Fasmac" Company (Kanagawa, Japan). The sequence of CpG ODN 2006 is 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3'. The CpG ODN was diluted in sterilized water and stored at -20°C before use.

Preparation of FITC-labeled CpG ODN loaded BNNSs

BNNSs solution and 3'-FITC-labeled CpG ODN solution were mixed in PBS buffer and continuously shaken at 4 °C for 4 h. In a typical experiment, 40 µL of BNNSs solution (1mg/mL in PBS) and 6 µL of FITC-labeled CpG ODN solution (100 µM stock in sterilized water) were mixed by rotation for 4 h.

Cellular uptake of BNNSs

Rhodamine B isothiocyanate (RBITC)-loaded BNNSs were used to investigate the cellular uptake and localization of BNNSs into the cells. $1x10^5$ 293XL-TLR9 cells were seeded in a 35mm perti dish with glass bottom and pre-incubated at 37 °C in humidified air containing 5% CO_2 for 48 h. Subsequently, RBITC-loaded BNNSs were added to the petri dishes at a final concentration of 10 ng/ml. Cells were fixed with 4 (v/v) % paraformaldehyde after culture and in some cases stained with DAPI and

antibody. Lysosome staining was achieved by incubation the cells with Rabbit polyclonal to LAMP1 (abcam, Cambridge, UK) and then Alexa Fluor 488 goat anti-rabbit IgG (H+L) secondary antibody (invitrogen, Calfornia, US). Free BNNSs, FITC-labeled CpG and FITC-labeled CpG loaded BNNSs were used in a parallel manner for analysis of CpG uptake into cells

NF-κB luciferase assay

For monitoring transient NF-κB activation, 293XL-hTLR9 cells were seeded into 24-well plates at a density of approximately $1.5x10^5$ cells per well and transiently transfected with pNiFty-luc (a TLR9-signaling reporter plasmid, purchased from Invivogen) and pGL4.74 (a CMV promoter -Renilla luciferase gene contained plasmid, as an internal control for variations in transfection efficiency, purchased from Promega (Wisconsin, US)) using transfection reagent, LyoVec (Invivogen). After a 24-hour culture, cells were stimulated with 0.3 μM of FITC-labeled CpG, free BNNSs, or FITC-labeled CpG loaded BNNSs respectively for another 24 hours. The luciferase activities were evaluated using the Dual-Luciferase assay system (Promega). Stimulated cells were lysed using a passive lysis buffer, and the resulting lysates were assayed for relative luciferase activity using a TD-20/20 luminometer (Promega) according to the manufacturer's instructions. The data were shown as fold increase in NF-κB activity over the medium control.

Others

The microstructures were analyzed using a JEOL-3000F high-resolution

field-emission transmission electron microscope operated at 300 kV. The differential interference contrast (DIC) and confocal fluorescence images were acquired by an SP5 confocal fluorescence microscope (CLSM; Leica microsystems, Germany).

Figure S1. TEM images of BN nanospheres.

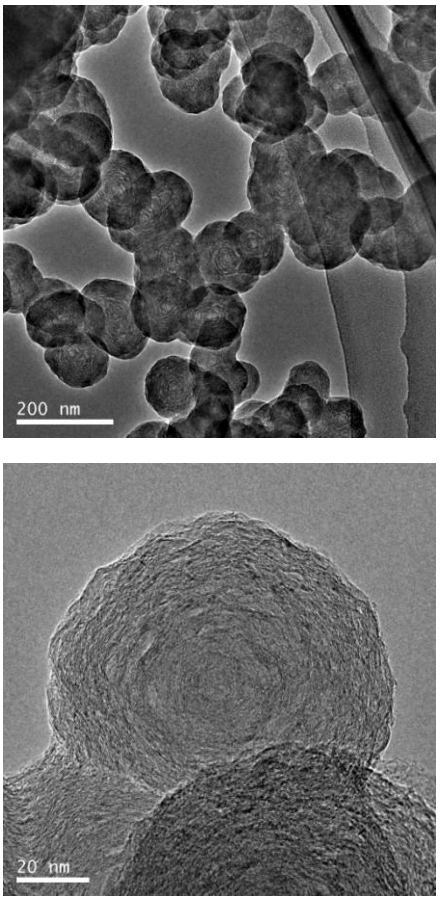

Figure S2. TGA analysis of a DNA sodium salt and a BN nanospheres-DNA sodium salt hybrid. The weight loss behavior of BN nanospheres-DNA sodium salt hybrid is very close to that of pure DNA sodium salt, which indicates that the weight loss is mainly due to oxidation of DNA sodium salt rather than BN nanospheres. In addition, DNA sodium salt itself has a final weight loss of 73%.