**Supplementary Material**

**Animals**

Animals were handled and housed in accordance with the Institutional Animal Care and Use Committee approved protocol. YAC128 transgenic mice (also known as FVB-Tg YAC128/53Hay/J line) Jackson Laboratories (Bar Harbor, ME, USA) were used because they express full length human mutant *HTT* and were the source of bone marrow mesenchymal stromal (BMMS) cell cultures. The mice had free access to water and food until euthanasia when bone marrow was harvested, as described below, for preparation of mesenchymal cell cultures that express mutant *HTT*.

**Cell culture**

Bone marrow mesenchymal stem (BMMS) cells were generated from 4-6 months-old transgenic YAC128 (also known as FVB-Tg YAC128/53Hay/J line) mice using previously described methods (10, 11). Mice were euthanized by cervical dislocation and sterilized in 70% ethanol than placed on a clean aluminum foil inside the biosafety cabinet. Hind limbs were detached from the body by cutting the caudal bone along with the femur. Skin and muscles along the bones were removed from the limbs. Foot and tibia underwent full removal. Both ends of the femur bones were cutoff with the scissors so that the marrow cells could be flushed out. The tip of a 26G needle (attached to a 5-ml syringe filled in with complete RPMI medium) has been placed into the proximal end of the femora to flush the marrow through the bone. The flush material was collected in a 50 ml sterile Falcone tube and passed through 22G needle 3-4 times to make single cell suspension. Tubes with cells underwent centrifugation (700×g for 5 min at RT) and medium replacement than cell were seeded in 25 cm2 flask at concentration 2 million/ml. On third and fifth day, two thirds of the medium were replaced with fresh growth medium. On seventh day, the cells reached 90% of conﬂuency and the treatment with 1 ml of 0.25% trypsin for 5 min at 37 °C was performed then BMMS cell cultures were used for functional experiments.

The cells were cultured in complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 10 mM HEPES, 1 mM sodium pyruvate, 1X Penicilline-Streptomicine 0.25 μg/ml Amphotericine B, 1X Non-essential Amino acids, 1X GlutaMAX, and maintained at 37°C and 5% CO2 under humid conditions.

**Reagents and materials**

Stock ampules (25 mg) of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, purity > 99%), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, purity > 99%) chloroform solutions were purchased from Avanti Lipids and stored at −20◦C. The stock ampules of CBD (1 mg), chloroform (purity > 99.8%), chloroform and methanol (purity ≥ 99.8%). Low molecular weight chitosan (CS) with molecular weight range of 50,000-190,000 Da and with 85% deacetylation, chitosan lactate (CSL) with molecular weight of 5000 Da, Lipofectamine 3000, Nile Red, Calcein and Hoechst 3334 were purchased from Sigma-Aldrich, MO. Mangafodipir Trisodium (MFDP) was purchased from U.S. Pharmacopeial Convention (Rockville, MD). Avanti Mini Extruder and polycarbonate membranes were purchased from Avanti Polar Lipids (AL, USA).

OptiMEM I reduced serum media, RPMI 1640, GlutaMAX, MEM non-essential amino acids solution, amphotericine B, Luria-Bertani (LB) broth, kanamycin, ampicillin, HEPES buffer solution, sodium pyruvate, penicillin-streptomycine, FBS, PBS (pH 7.4), trypan blue (0.4 %), and trypsine-EDTA were obtained from Invitrogen (Carlsbad, CA, USA). Tissue culture flasks (75 cm2, 25 cm2), 96-well plates were used from Alkali Scientific inc. (ASi, FL. USA), and 24- and 6-well tissue culture plates from CytoOne (USA Scientific, US).

Zyppy plasma Miniprep kit and ZymoPure plasma Maxiprep kit were purchased from Sigma (St. Louis, MO, USA).

TaqMan™ Fast Advanced Master Mix and primers for qPCR were obtained from ThermoFisher, including HTT (assay ID: Hs00918174\_m1) and PPIB as a reference gene (assay ID: Mm00478295\_m1).

Mouse IL-6 ELISA kit, lipopolysaccharide (LPS) from *Escherichia coli* serotype 0111:B4 and Dexamethasone were purchased from Sigma (St. Louis, MO, USA).

All other chemicals and reagents used were of analytical grade. Ultrapure distilled DNase, RNase free water purchased from Thermo Fisher Scientific Inc. (Waltham, MA) was used for all experiments.

**Plasmid DNA**

*E. coil* transformed with plasmid DNA (pmCherry) were inoculated into LB broth media with 1µl for 1ml medium (Kanamycin 50 mg/ml, Ampcillin 100 mg/ml) for 20 - 24 h at 37oC. Plasmid DNA was isolated by Zyppy plasmid Miniprep kit. ZymoPure plasmid Maxiprep kit was used to purified transfection grade plasmid DNA. Plasmid DNA concentration was determined by UV-Vis measurements by NanoDrop 23300. The purity of pDNA was over 95%.

**siRNAs**

Three different siRNA compounds were packaged into nanoparticlesor liposomes. Silencer select siRNA s6491 was purchased from ThermoFisher Scientific. Novel siRNA targeting exon 30 of *HTT* mRNA (E30) was designed by using the RefSeq sequence NM\_002111.8. This siRNA was designed in our laboratory with the use of theoretical basics, web-based tools, and open-access databases. E30 was labeled with Cy3 to assess the efficiency of siRNA uptake. The scrambled siRNA, which has no homology to any known mammalian genes including *HTT* was used as the non-target control (NTC). Both E30 siRNA and NTC were synthesized, modified, and purified by Dharmacon Inc., a [Horizon Discovery](https://www.horizondiscovery.com/) Group Company.

**Nanoparticle preparation**

Polymeric NPs were fabricated via ionotropic gelation of chitosan and nucleotide solution as described previously (3). The optimal NPs were formulated to obtain a final amine-to-phosphate (N/P) ratio of 2:1 (CS/oligonucleotide) and 1:1 (CSL/ oligonucleotide) respectively. Cross linking agent MFDP (1-10 μg/ml) was added dropwise under continuous stirring at room temperature.

**Liposome preparation**

The liposomes were prepared according to the classical film hydration method. Phospholipids DPPC and DOPC together with CBD stock solutions each dissolved in 2:1 (v/v) chloroform/methanol mixture were blended in amber glass bottle at 5:2.5:1 mol% proportions. Nile Red dissolved in chloroform was used at stock concentration 100 μg/ml. A thin lipid film was obtained by evaporation of organic solvent under the vacuum. To obtain liposomal suspension the lipid film was hydrated in distilled, deionized water on magnetic stirrer for 30 min at 1100 rpm and at moderate heat. Liposomes were subjected to sonication following by extrusion (11 times) through 200 nm polycarbonate membranes (Whatman) performed with Avanti Mini Extruder (Avanti Polar Lipids) to reduce their size.

**Diagram

Description automatically generated**

**Fig. S-1** Diagram depicting preparation of DNA-containing liposome

**Hybrid nanoparticles preparation**

Hybrid nanoparticles were prepared by fusion of liposomes and nanoparticles by adding appropriate amount of liposomes solution to nanoparticle solution at mass w:w ratio 1:2. The mixture was stirred for 1 h at 1100 rpm above phase transition temperature. The final HNP composition: CS/CSL (40%), DPPC (24%), nucleic acid (21%), DOPC (13%) and CBD (2%).

Diagram

Description automatically generated with low confidence

**Fig. S-2** Diagram depicting preparation of multifunctional hybrid nanoparticle

**Assessment of Lamination Efficiency**

Lamination efficacy (LE%) of nanoparticles with lipid layer was measured by fluorescence detection of hydrophilic and hydrophobic dyes. The pDNA contained nanoparticles were stained with fluorescent dye Hoechst 33342 (excitation 350 nm, emission 462 nm) and liposomes were stained with Nile Red (excitation 552, emission 639 nm). Hoechst 33342 stock solution was added to pDNA before encapsulation. The fluorescence readings of samples were performed in 96-well plates by using Synergy H1 Hybrid Multi-Mode Reader (BioTek Instruments, Vermont, USA). LE% was quantified with equation:

LE% = 100×(1 - F1/F2),

Where, F1 denotes the changes in Hoechst 33342 fluorescence and F2 represents the changes in fluorescence of Nile Red.

**Particle size and zeta potential.**

Size and ζ-potential of nanoparticles, liposomes and lipid-coated nanoparticles were measured using a Zetasizer (Nano-ZS, Malvern Instruments Ltd., UK). Based on liposomal size, large unilamellar vesicles (LUVs, >100 nm) were obtained (7). The N/P ratios required for the full condensation of DNA into nanocarrier were confirmed by gel retardation assay.

**Cell viability study**

BMMS cells were dispensed in 24-well plates at a density of 1×105 cells per well and treated with pDNA loaded into NPs and HNPs. Lipofectamine 3000 was used as a control. Treatment was performed with following doses of pDNA: 0, 2, 4, 8, 10 μg/ml for 24 hours. Cells were detached by trypsinization, and the number of viable cells was counted by using a 0.4% Trypan blue staining reagent. The viability of control (untreated cells) was considered as 100%.

**Transfection study**

BBMS cells were seeded onto 24-well plates at a density of 1×105 cells per well and incubated for 48 h before transfection. NPs, HNPs and Lipofectamine3000-based Ls containing pDNA were added in triplicates to the wells with OptiMEM medium and the plates were gently swirled (dose 4 μg/ml). As a positive control, a commercial transfection reagent Lipofectamine3000 has been used. Fresh growth medium was added to the wells after 6 h incubation at 37˚C, 5% CO2. The cells were left in the incubator for 24h before they were further assayed and monitored for 5 days. Each experiment was performed in triplicates and the results are presented as mean of these experiments. The number of positive cells within a transfected cells population was monitored and determined through fluorescent digital microscope (Keyence VHX-700). Transfection efficiency was measured by the image analysis software (ImageJ)

**Cellular uptake**

The ability of NPs and HNPs to deliver Cy3-labelled siRNA E30 into BMMS cells was investigated. A 6-well plate was prepared with the BMMS cell line (2.5×105 cells per well) and cultured at 37°C for 48 h in regular medium. The culture medium was changed for OptiMEM for transfection performance. E30 loaded nanoformulations were diluted in OptiMEM and added to the cells (2.5 μg/ml). After 6 h incubation cells were washed twice with phosphate-buffered saline (PBS) with following incubation in regular medium. To stain cells and nucleus the working solution of indicators in complete medium (1 μM calceine and 1 μM Hoechst 33342) was added into each well and incubated for 30 min at room temperature. Cells were studied without fixation by fluorescence microscopy, the imaging conditions were kept constant for the observation of the different samples, and images were taken with Keyence VHX-700 digital microscope.

***HTT* gene silencing *In vitro***

To investigate the efficacy of each siRNA to downregulate expression of the target gene BMMS cells were seeded onto T25 cm2 cell culture flasks at a density of 4x106 cells per flask and incubated for 48 hrs before transfection. CSL-based NPs, HNPs and Lipofectamine3000-based Ls loaded with siRNAs were diluted with OptiMEM medium and added to the flasks. The amount of siRNA was 10 μg per flask (2.5 μg/ml). The cells were left in the incubator for 24h at 37˚C, 5% CO2 before they were further assayed. The experimental results are presented as mean.

**Evaluation of *HTT* silencing**

Total RNA was extracted with the use of TRIzol Reagent and PhasemakerTM tubes (Fisher Scientific). The RNA was reverse transcribed using Invitrogen™ SuperScript™ III Reverse Transcriptase kit with Invitrogen™ Oligo(dT) 20 Primer (Fisher Scientific).

RNA was reverse transcribed using Invitrogen™ SuperScript™ III Reverse Transcriptase kit with Invitrogen™ Oligo(dT) 20 Primer (Fisher Scientific). Levels of mRNA expression were measured with QuantStudio3 (ThermoFisher). TaqMan™ Fast Advanced Master Mix (ThermoFisher,. MA) was used for quantitative Real-Time PCR (qPCR) carried out in accordance with manufacturer protocol. Expression levels for huntingtin mRNA were normalized to PPIB (peptidylprolyl isomerase B) mRNA levels. Primers for qPCR were obtained from ThermoFisher, including HTT (assay ID: Hs00918174\_m1 and assay ID: Mm01213820\_m1) and PPIB (assay ID: Mm00478295\_m1).

**Anti-inflammatory potential of CBD loaded liposomes**

BMMS cells were seeded onto 24-well plates at a density of 1×105 cells per well and incubated for 48 h. Cells were stimulated by replacing culturing medium with a similar volume of OptiMEM medium containing 1 μg/ml of lipopolysaccharide (LPS); or LPS plus 1 μg/ml dexamethasone (DEX); or LPS in combination with Ls loaded with CBD (1 μg/ml). All the different conditions were tested in triplicates. The conditioned medium was collected after 48 h of incubation at 37oC and kept frozen until assayed for cytokines by Mouse IL-6 ELISA.