Supporting information

An Anti-P0 Antibody-Conjugated Nanoscale Contrast Agent Targeting Myelin Sheath for Intraoperative Visible Delineation of Cranial Nerves

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Experimental

1. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. ^{1}H NMR (400 MHz) of BMPU molecules was conducted on a Bruker AV II-400 MHz

spectrometer. Tetramethylsilane (TMS) was used as an internal standard in dimethyl sulfoxide (DMSO)-d_6.

2. Fourier transform infrared (FT-IR) spectroscopy. One milligram of BMPU in DMAc was pressed into potassium bromide (KBr) pellets. FT-IR spectra were obtained on a Nicolet 6700 spectrometer (Thermo Electron Corporation, USA) in a wavelength range between 4000 and 600 cm^{-1} with a resolution of 4 cm^{-1}.

3. Gel permeation chromatography (GPC). To determine the molecular weights and molecular weight distributions of BMPU molecules, GPC was performed on a PL-GPC 220 instrument (Polymer Laboratory Ltd., UK) using N,N-dimethylformamide (DMF)/LiBr as the eluent and polymethyl methacrylate (PMMA) as the reference. The sample concentration was 5.000 mg/mL, and the flow rate was 1.000 mL/min.

4. Transmission electron microscopy (TEM). A drop of micellar solution stained with 1% (w/v) phosphotungstic acid was placed on a copper grid with Formvar film, and the liquid removed from the sample by blotting and air-drying before conducting the measurement. TEM was then performed on a Hitachi model H-600-4 transmission electron microscope with an accelerating voltage of 75 kV. TEM showed dispersed individual particles with regular spherical shapes for both the CB-G00-Ab and CB-G50-Ab NPs (Figure S1).

5. Dynamic light scattering (DLS). The sizes and zeta potentials of BMPU micelles were measured with a Zetasizer Nano ZS dynamic light scattering (DLS) instrument (Malvern, UK) at 25 °C and an angle of 90°. The zeta potentials of all the five BMPU micelle samples were compared, and the sample that possessed the appropriate charge density was used for further conjugation of antibodies and loading of CB. G00 was used as the GQA-free control.

6. Pyrene fluorescence spectrum. To determine the critical micelle concentration (CMC), fluorescence measurements were carried out using pyrene as a hydrophobic probe. Samples were prepared by adding known amounts of pyrene (5.0×10^{-6} mol/L) in acetone to a series of vials, and the acetone was then evaporated. Next, different amounts of micelle solution were added to the vials. The final pyrene concentration was 5.0×10^{-7} mol/L. All the samples were equilibrated upon shaking with ultrasound for 3 hours and incubated overnight at room temperature. Steady-state fluorescence spectra were acquired using an F-7000 FL spectrophotometer with bandwidths of 5.0 nm for excitation and 2.0 nm for emission. For the excitation spectra, λ_{ex} was 334.0 nm, and for the emission spectra, λ_{em} was 373.0 nm.

7. Enzyme-linked immunosorbent assay (ELISA). The amount of P0-specific antibodies conjugated to G00-Ab and G50-Ab micelles was measured by an

ELISA following the instructions of the anti-P0 antibody ELISA kit (CUSABIO, USA). Samples were lyophilized and then diluted to 0.1 mg/mL with deionized water to satisfy the detection range of the kit (1 to 1000 ng/mL).

8. UV absorption spectroscopy. The amount of CB loaded inside NPs was determined by a UV/Vis spectrometer (UV-1800PC, Shanghai Mapada Instruments Co., Ltd., China). The detection of CB was performed by UV absorption spectroscopy at a wavelength of 610 nm (Figure S2). The loading content (LC) and encapsulation efficiency (EE) were calculated based on the following equations:

LC (%) = mass of the drugs in the micelles / total mass of the loaded NPs × 100%, and

EE (%) = mass of the drugs in the NPs / initial amount of loaded drugs × 100%.

9. Intravenous injection of NPs.

Six BALB/c mice were divided into 2 groups. CB-G00-Ab and CB-G50-Ab were slowly injected through the tail veins of mice at a dose of 50 μL/g. After 30 minutes, the mice were euthanized. The sciatic, brachial and trigeminal nerves were exposed for observation.

Tables

Table S1. Theoretical composition of biodegradable multiblock polyurethanes with various amounts of GQA in chain extenders.

Sample	Molar ratio of LDI/PCL/m-PEG/Chain extenders	Molar ratio of chain extenders Tripeptide/GQA/PDO
G00	4.32/1.60/0.80/2.00	1.00/0.00/1.00
G25	4.32/1.60/0.80/2.00	1.00/0.25/0.75
G50	4.32/1.60/0.80/2.00	1.00/0.50/0.50
G75	4.32/1.60/0.80/2.00	1.00/0.75/0.25
G100	4.32/1.60/0.80/2.00	1.00/1.00/0.00

Figures

Figure S1. TEM images of CB-BMPU-Ab nanoparticles prepared from G00 (Left) and G50 (Right). Scale bars, 100 nm.

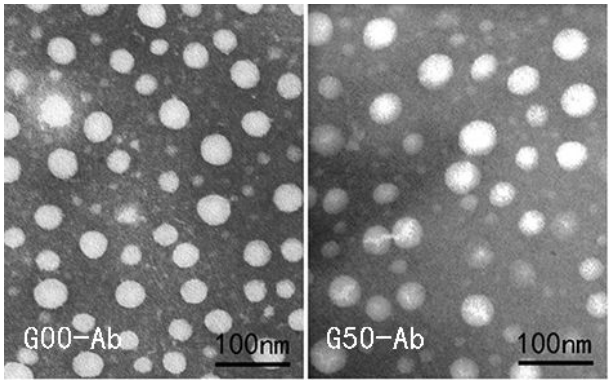

Figure S2. The absorption-concentration standard curve of CB. The NPs were diluted to 0.1mg/mL.

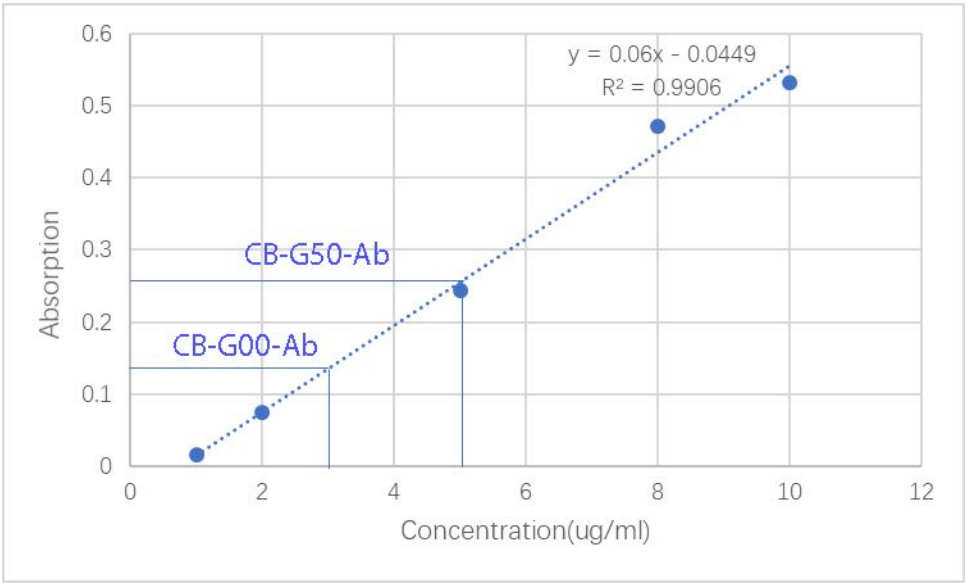

Figure S3. The anti-P0 concentration-OD standard curve of ELISA assay.

OD = optical density.