Electronic Supplementary Information (ESI)

Bacteria mimics bearing carbohydrates, ologodeoxynucteotides and designed shapes

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Experimental Section

Materials:

Multi-walled carbon nanotubes (MCNT) was purchased from Macklin (＞95%, ID:5-10 nm, OD:10-20 nm, length: 0.5-2 μm), Dimethyl sulphoxide (DMSO) (analytical reagent, Shanghai Chemical Reagent Co. Ltd) was dried by 4 Å molecular sieves before use. Chloroauric acid hydrated ($HAuCl_4 \cdot 4H_2O$) was purchased from Shanghai Shi Wu Chemical Reagent Science and Technology Co, LTD. CpG-NH_2 (5'-amine-TCCATGACGTTCCTGACGTT-3') was bought from Shang Hai Sangon Biotech. RPMI-1640 medium, fetal bovine serum (FBS) and penicillin-streptomycin solution were purchased from Gibco (Grand Island, NY, USA). PE anti-human CD86 and CD206 antibodies were from ThermoFisher Scientific (Lithuania, EU). Polydopamine microsphere (PDA)[1], 2-Cyanoprop-2-yl-α-dithionaphthalate (CPDN)[2],

N-3,4-dihydroxybenzenethyl methacrylamide (DMA)[3], 2-(methacrylamido) glucopyranose (MAG)[4], glycopolymer PDMA-*co*-PMAG[5] were synthesized and characterized according to previously reported work. Fluorescein-labeled ConA (ConA-FITC) bought from Sigma-Aldrich were used directly. SGAs (Spherical Glycoadjuvants) were prepared as reported previously[6]. Deionized water, purified to a minimum resistivity of 18.25 MΩ·cm by a UPH water purification system, was used in all experiments. All other chemicals were commercially available and used without further purification, unless specified.

Characterization:

^{1}H NMR spectra were collected with a Bruker nuclear magnetic resonance instrument (300 MHz) and using tetramethylsilane (TMS) as the internal standard at room temperature. Zeta potential and size were measured by Dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Instruments Ltd). Transmission electron microscopy (TEM, Tecnai-G20, 120 kv, FEI) equipped with an energy-dispersive X-ray (EDS) spectrometer was using in this work. FT-IR spectra were obtained by a Nicolet 6700 via potassium bromide tableting method (ThermoFisher Scientific, USA). The quantitative analysis of gold and phosphorus was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, iCAP 7200 HS Duo, ThermoFisher Scientific). The confocal fluorescence images were captured with an inverted confocal laser scanning microscope (Zeiss, LSM 710) equipped with a 60x oil objective. Flow cytometry was performed on a BD FACSVerse™ system and the data were analyzed using FlowJo.

The synthesis of PDMA-*co*-PMAG via sunlight-induced RAFT polymerization:

MAG (0.12 g, 0.486 mmol), DMA (36 mg, 0.163 mmol), CPDN (0.7 mg, 0.0026 mmol) were dissolved by 0.8 ml DMSO inside 5 ml ampule with a magnetic stirring bar. The above solution was purged with argon for 15 min to deoxidize. Then the ampoule bottle was flame-sealed under argon atmosphere and placed under the simulated sunlight at room temperature. At the designed time, the ampoule bottle was

opened and the mixture was diluted and then precipitated into 250 mL methanol. The product was obtained by filtration and dried to keep constant weight under vacuum.

The synthesis of SGAs:

Firstly, dissolve 0.76 mg glycopolymer and 60 μg CpG ODN in 500 μL ultrapure water. Afterward, 10 μL $HAuCl_4$ aqueous solution (1% Wt) was added into the above solution and reacted overnight on the shaker at room temperature. The prepared functional gold nanoparticles were collected by centrifugation (7000 r/min×10min), and were stored at 4°C for further use.

The synthesis of CpG@MCNT and CpG@PDA:

2.4 μL CpG ODN (3 mg/ml) were added to 20 μL MCNT/PDA (0.05mg/ml) and incubated for 1h. After removing free CpG ODN by centrifugation (7000 r/min×5min), they were collected and re-suspended in ultrapure water for further use, which were named as CpG@MCNT and CpG@PDA, respectively.

The synthesis of bacteria mimics SGAs@MCNT and SGAs@PDA:

60 μL SGAs were added to 20 μL MCNT/PDA (0.05mg/ml) and incubated for 1h. Free SGAs were removed by centrifugation for 5 min (7000 r/min×5min), and finally, the products were collected and re-suspended in ultrapure water for further use, which were named as SGAs@MCNT and SGAs@PDA, respectively.

The interaction between the bacteria mimics SGAs@MCNT and SGAs@PDA with Con A-FITC:

SGAs@MCNT or SGAs@PDA was dispersed in 60 μL HEPES buffer solution (10 mM, pH 7.5, containing 1 mM Ca^{2+}, 0.15 mM Na^{+}, and 0.01 mM Mn^{2+}), then 20 μL Con A-FITC (1mg/ml) were added into the above solution. Afterward, the mixed solution was incubated in dark condition for 10min, separated the free Con A-FITC by centrifugation (7000 r/min×5min) and re-suspended in HEPES buffer solution, and then a drop was taken to a glass slide for characterization with an inverted confocal laser

scanning microscope (Zeiss, LSM 710) equipped with a 60x oil objective.

Cell Culture:

HeLa cells were cultured in DMEM medium, and U937 cells were cultured in RPMI-1640 medium. All the above-mentioned media were supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were incubated at 37 °C with 5% CO_2 in a water-saturated chamber.

Co-culture of U937 cells and different bacteria mimics:

U937 cells were seed on 48-well plate at a density of 3×10^4 cells per well in RPMI-1640 medium. All media were supplemented with 10% FBS, 100U/mL penicillin and 100 μg/mL streptomycin. Then the cells were incubated respectively with CpG@MCNT, CpG@PDA, SGAs@MCNT and SGAs@PDA at 37°C in a water-saturated chamber (5 % CO_2) for appropriate times, finally images were captured by IncuCyte ZOOM (Essen Bioscience).

Analysis of CD86 and CD206 expression on U937 by flow cytometry：

After co-culture of U937 cells with different bacteria mimics for 27h in a 6-well tissue culture plate, the cells were collected and centrifuged at 1000 rpm for 5 min. Afterwards, the cells were washed twice with PBS and then labeled with PE anti-human CD86 and CD206 antibody for 40 min. Finally, the cells were re-suspended in 0.5 mL PBS and to be analyzed by flow cytometry.

Co-culture of U937 cells and different bacteria mimics in the presence of Hela cells:

HeLa cells were seeded in a 48-well plate at a density of 3×10^4 cells per well in complete DMEM medium and incubated for 12 h. And then U937 cells were seeded on HeLa-adhered plates (U937 cells : Hela cells = 1 : 1) and incubated with SGAs@MCNT and SGAs@PDA for appropriate times.

Analysis of CD86 and CD206 expression on U937 in the presence of Hela cells by flow cytometry：

After co-culture for 27 h, the cells were washed with PBS, lifted with trypsin solution and centrifuged at 1000 rpm for 5 min. The cells were then washed twice with PBS and subsequently labeled with PE anti-human CD86 and CD206 antibodies for 40 min. Finally, the cells were re-suspended in 0.5 mL PBS and analyzed by flow cytometry.

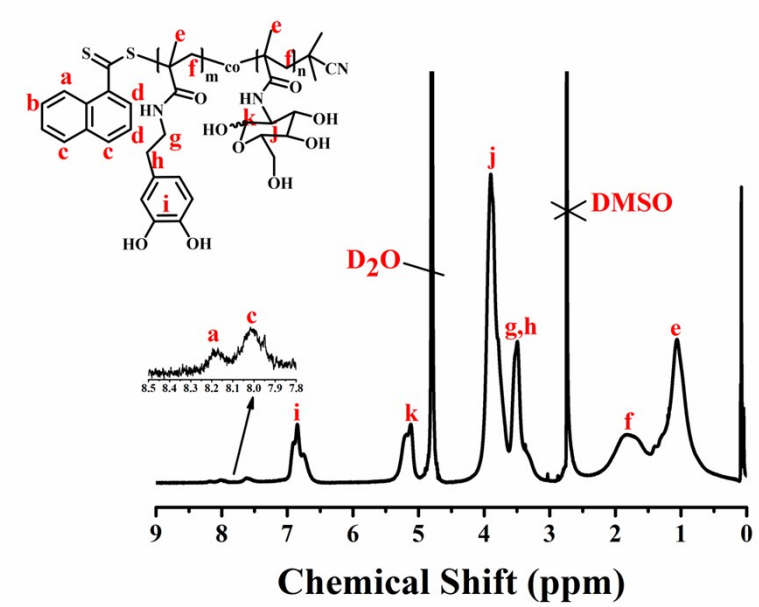

Figure S1. ^{1}H NMR spectrum of glycopolymer PDMA$_{19}$-*co*-PMAG$_{57}$ ($M_{n(NMR)}$ = 18500 g/mol).

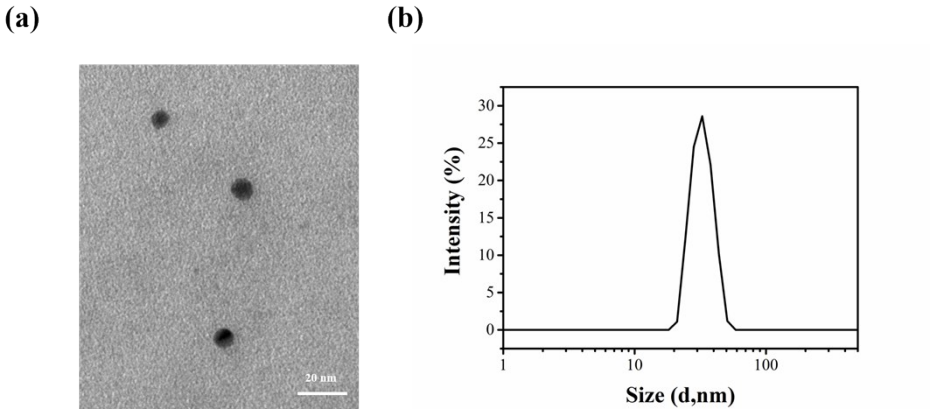

Figure S2. (a) TEM image of SGAs; (b) Hydrodynamic diameter of SGAs (Z-average$_{(d,nm)}$ = 25.6 nm, PDI = 0.318).

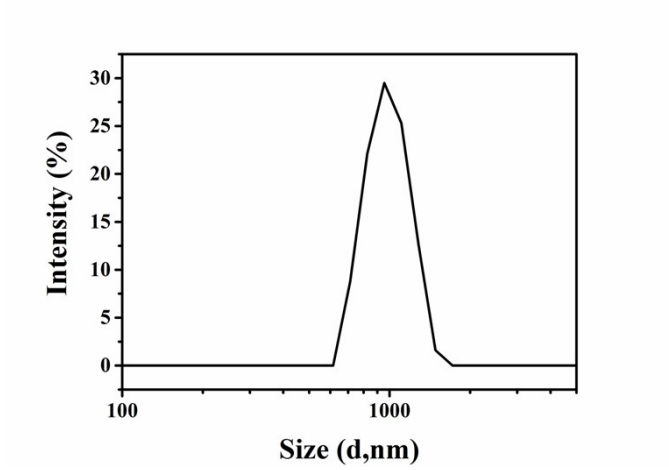

Figure S3. Hydrodynamic diameter of PDA microsphere (Z-average$_{(d,nm)}$ = 1530 nm, PDI = 0.519).

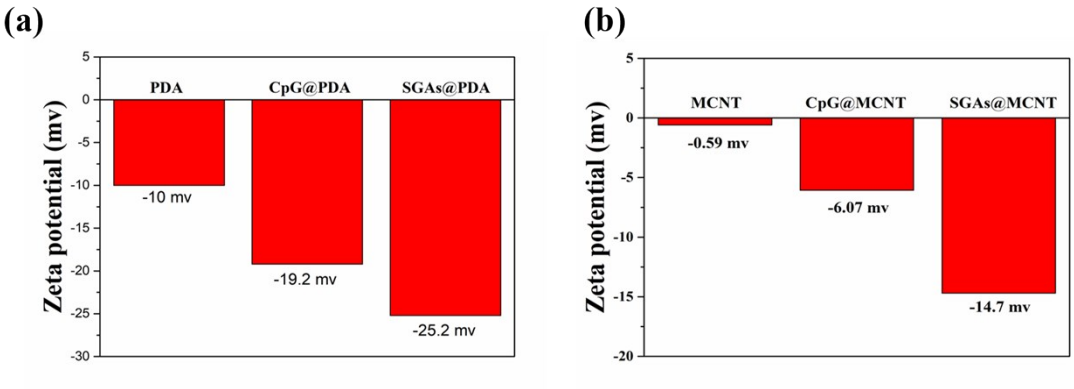

Figure S4. Zeta potential of (a) PDAs before and after modification with CpG and SGAs; (b) MCNTs before and after modification with CpG and SGAs.

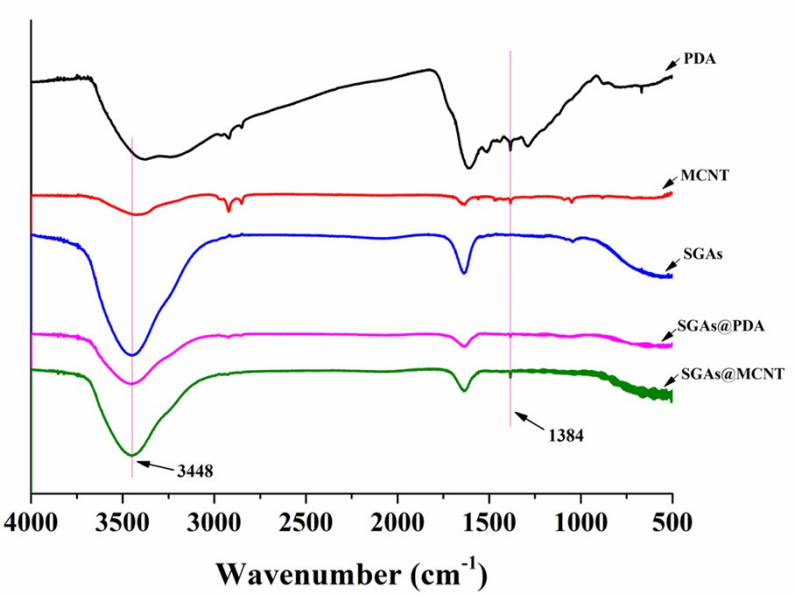

Figure S5. The FT-IR spectra of PDA, MCNT, SGAs, SGAs@MCNT and SGAs@PDA

Bacteria mimics	Mass content of scaffold (MCNT or PDA) (mg)	Mass content of Au (mg)	Mass content of P (mg)	Molar content of Au (μmol)	Moalr content of P (μmol)
SGAs@MCNT	1	0.396	0.046	2	1.5
SGAs@PDA	1	0.858	0.086	4.3	2.8

Figure S6. The content of Au and P in each milligram for scaffold SGAs@MCNT and SGAs@PDA calculated by ICP-AES.

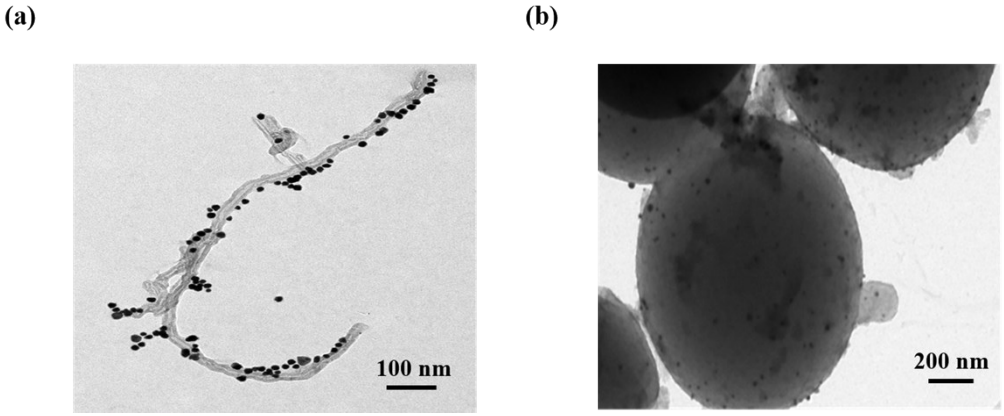

Figure S7. TEM images of the bacteria mimics. (a) SGAs@MCNT; (b)SGAs@PDA.

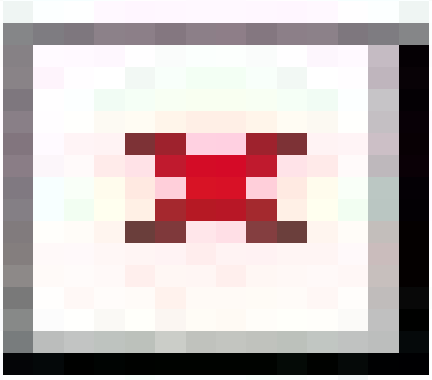

Figure S8. Confocal fluorescence images of (a) SGAs@MCNT (Scale bar = 2 μm); (b) SGAs@PDA after interaction with ConA-FITC (Scale bar = 1 μm).

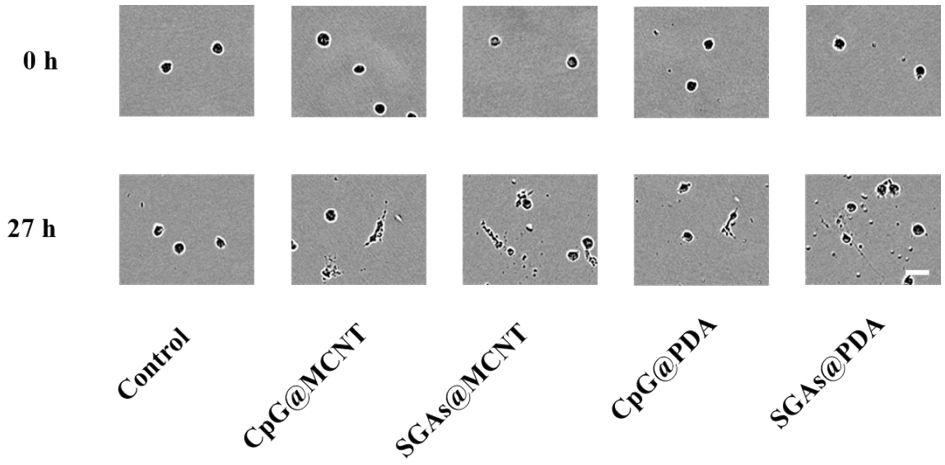

Figure S9. Activation and polarization of U937 cells treated with different bacteria mimics monitored by IncuCyte ZOOM (CpG content in each group was 3.6 μg/ml, Scale bar = 20 μm).

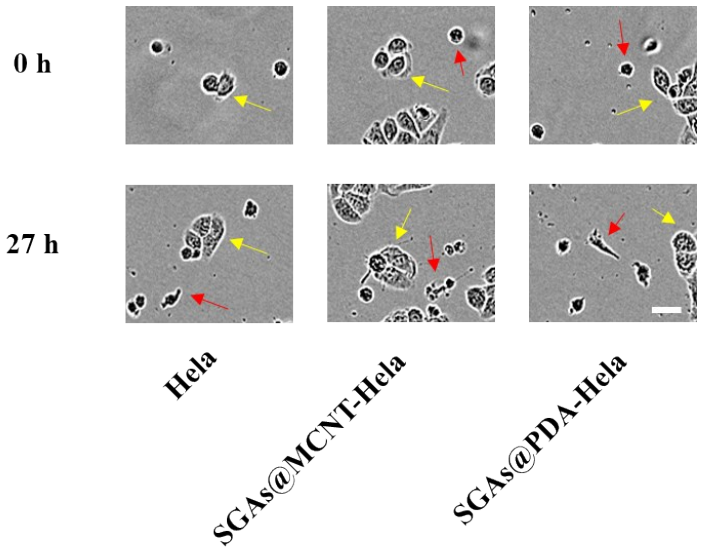

Figure S10. Activation and polarization of U937 cells treated with different bacteria mimics in the presence of Hela cells. Red arrows point toward U937 cells, while the yellow arrows point toward adhered HeLa cells monitored by IncuCyte ZOOM (CpG content in each group was 3.6 μg/ml, Scale bar = 20 μm).

References

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