Synthesis and application of glycoconjugate-functionalized magnetic nanoparticles as potent anti-adhesin agents for reducing enterotoxigenic *Escherichia coli* infections

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Synthesis of magnetic nanoparticles: Reprinted from (46) - Reproduced by permission of The Royal Society of Chemistry

The 7.2nm magnetic nanoparticles, synthesized using thermal decomposition of iron(III) acetylacetonate (2mmol), 1,2-hexadecanediol (10mmol), olylamine (4mmol), benzyl ether (20ml), and 6nm iron oxide seeds were added and stirred under a nitrogen flow and brought to 200°C for 1hr to get rid of any moisture. Finally the reaction was brought to reflux for 30mins under a nitrogen head. The particles were purified by precipitation of ethanol and characterized using TEM and DLS.

Modification of magnetic nanoparticles: Reprinted from (46) - Reproduced by permission of The Royal Society of Chemistry

The magnetic nanoparticles were modified by first dissolving alkyne-PEO-nitroDOPA (200 mg, 0.04mmol) into 10ml of chloroform followed by the slow addition of 1ml (2mg/ml) of magnetic nanoparticles, which were also dispersed in chloroform, while sonicating over 30 mins. The solution was allowed to stir overnight. The particles were then purified by precipitation with hexane, centrifuged to separate particles from solvent. They were then dispersed in ethanol and subsequently precipitated using hexane and separated via centrifugation to collect particles. Finally, the particles were dispersed in deionized water and dialyzed for 3 days.

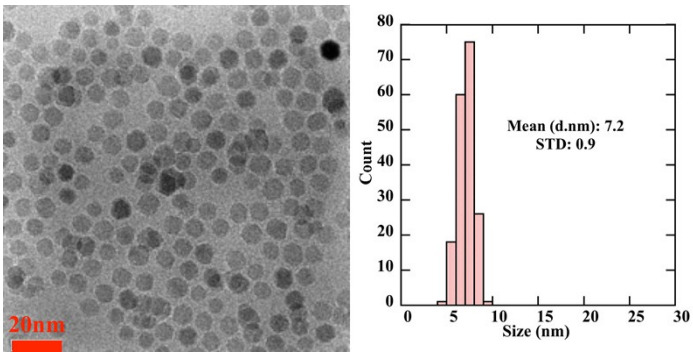


Figure S1. TEM micrograph of oleylamine coated magnetic nanoparticles on the left and histogram of particles size distribution on the right. Reprinted from (46) - Reproduced by permission of The Royal Society of Chemistry

Dynamic Light Scattering (DLS) and Zeta-Potential:

DLS was performed on the PEO-coated and GM3-coated magnetic nanoparticles to determine their hydrodynamic radius. The nanoparticle suspensions were diluted in water and placed into a cuvette. The readings were taken at 25°C three times using Malvern Zetasizer Nano ZS to determine the intensity average size distribution and z-average diameter. Zeta-potential measurements of these nanoparticle suspensions were also determined using the same instrument. The suspensions were diluted with water and added into zeta-cell and measurements were taken three at 25°C.

Type of MNPs	DLS (Z – Avg) (d. nm)	Zeta-Potential
PEO-MNPs	77.31	-12.1
GM3-MNPs	80.46	-42.0

Table S1: DLS and Zeta-Potential measurements of MNPs after functionalizing PEO polymer and GM3 molecule.

Inductively coupled plasma mass spectroscopy (ICP-MS):

The nanoparticle concentration in PEO-MNPs and GM3-MNPs was determined by performing ICP-MS (Thermo-Scientific MS XSeries 2). The nanoparticle suspensions were treated with 2% nitric acid solution in a 15 ml centrifuge tube and subsequently measurements were taken.

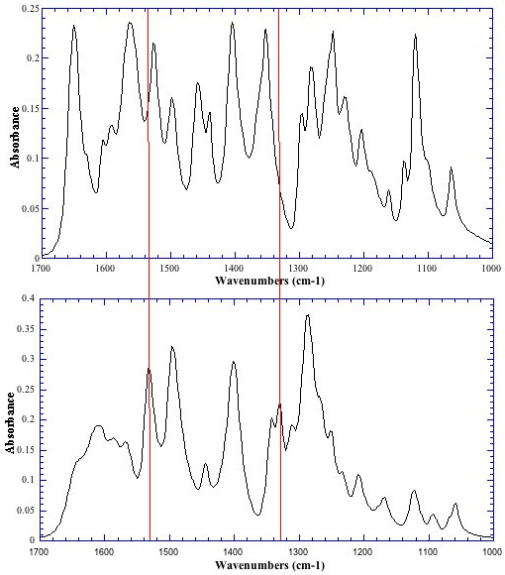
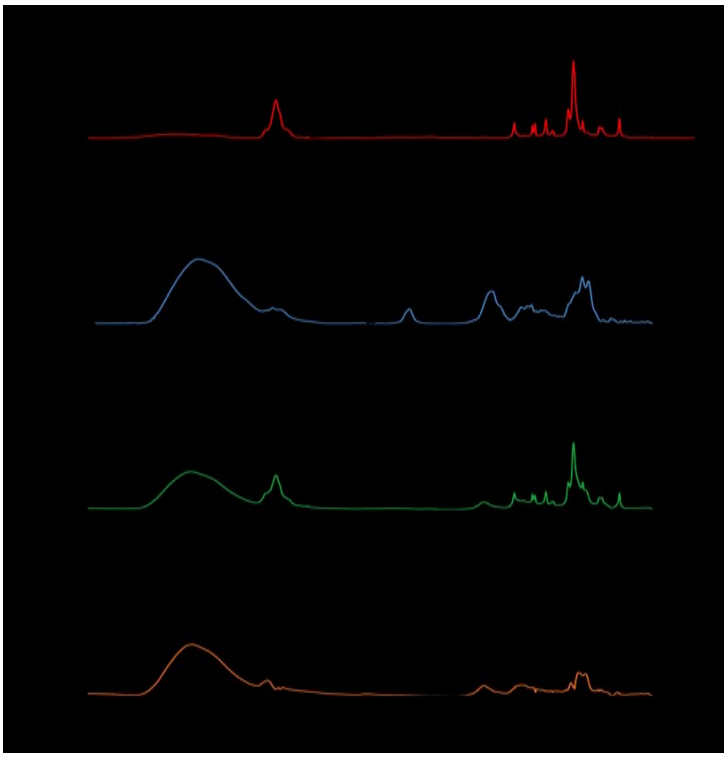


Figure S2. FTIR of DOPA (Top) and NitroDOPA (Bottom). Symmetric and asymmetric stretching from the NO_2 peaks at 1330 and 1532 cm^{-1}. Reprinted from (46) - Reproduced by permission of The Royal Society of Chemistry.



Figures S3: FTIR spectroscopy of A.) Magnetite particles modified with the alkyne PEO stabilizer, B.) The Neu5Ac(α2-3)Gal(β1-4)Glcβ-sp targeting moiety prior to "clicking", C.) The resulting complex of magnetic nanoparticles with the Neu5Ac(α2-3)Gal(β1-4)Glcβ-sp moiety, and D.) The resulting spectrum of subtracting spectrum C by A indicating the successful conjugation of the Neu5Ac(α2-3)Gal(β1-4)Glcβ-sp to targeting molecule.

Fourier transform infrared (FTIR) spectroscopy assisted microscopy was done using a Thermo-Nicolet Magna 550 FTIR spectrometer equipped with a Thermo-NicPlan FTIR microscope. Scan number was 16 for each sample including background. Background was collected on a clean germanium plate. Samples were prepped by taking the water suspensions/solution and casting a small drop on a germanium plate. The samples were then set under a heat lamp for 10-15 minutes to dry, resulting in a thin film from which spectrum could be collected using an FTIR microscope.

As seen in Figure S3, peaks at 1116cm^{-1} (C-O stretching) and 843 cm^{-1} (O-CH_2-C rocking) in Spectrum A are indicative of poly(ethylene oxide) (PEO). Peaks at 3393cm^{-1} (O-H stretching), 2113cm^{-1} (N=N=N stretching), 1072cm^{-1}+ 1035cm^{-1} (C-O stretching) in spectrum B indicate both the azide functionality and the polyglyceride nature of the Neu5Ac(α2-3)Gal(β1-4)Glcβ-sp compound. In spectrum C, the disappearance of the azide peak at 2113cm^{-1} with the appearance of the OH peak at 3387cm^{-1} and C-O peak from the Neu5Ac(α2-3)Gal(β1-4)Glcβ-sp at 1046cm^{-1} and the PEO stretching and rocking peaks at 1116cm^{-1} and 843cm^{-1}, shows that the azide-alkyne cycloaddition was successful and went to completion with no excess Neu5Ac(α2-3)Gal(β1-4)Glcβ-sp in solution. Spectrum D further supports the success of the reaction as a subtraction of A from C yields a spectrum similar to B,

the only major difference being the absence of the azide peak at 2113 cm^{-1}. This is to be expected as the azide peak disappears after the cycloaddition occurs reacts.

TEM Imaging of GM3-MNPs and *EC* K99:

TEM imaging was performed to evaluate the specific binding interactions between GM3-MNPs and *EC* K99. GM3-MNPs were added to EC K99 (5 x 10^7 CFU/ml, suspended in 1X PBS) in an eppendorf tube for 30 minutes at room temperature with gentle shaking. The mixture was centrifuged at 7000 x g for 5 minutes to spin down the bacterial cells along with adherent GM3-MNPs. The supernatant containing unbound GM3-MNPs was removed and the pellet was washed thrice with 1X PBS in repeated centrifugation cycles. This mixture was then fixed in cacodylate-buffered glutaraldehyde (3.5%, pH ~7.4) for 10 -12 hours at 4°C. Subsequently, 3 µl of this mixture was dropped onto a carbon-coated copper grid and allowed to air-dry for 30 minutes. Later, the grids were stained with 2% uranyl acetate solution (3 µl) for 5 minutes and blotted dry with filter-paper (Whatman #4). TEM images were taken on Hitachi H7600 at 120 kV power and magnification ranging from 10000X to 100000X.

Culturing of CCD-18Co (ATCC CRL-1459):

CCD-18Co Human colon cells (normal) were procured from American Type Culture Collection (ATCC) and grown on 50 cm^2 tissue-culture flask (Corning, NY) in the presence of Eagle's Minimum Essential Medium (EMEM) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. EMEM was supplemented with 2 mM L-Glutamine, non-essential amino acids, fetal bovine serum (final concentration – 10%), 100 UI/ml penicillin G, and 100 µg/ml streptomycin. For determining the cytotoxicity of GM3-MNPs, cells between passage generation of 12 and 20 were used. 1.5 x 10^4 cells/well were seeded (in triplicates) in 96-well culture-plates at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After 24 hours, varying concentration of GM3-MNPs were added to the cells and incubated for further 24 hours. Next day, MTS asaay was performed according to manufacturer's protocol and the plate was read at 490 nm optical density to measure the absorbance of the formazan product using a microplate reader (Thermo Scientific Multiskan™ FC).