Supporting information

Hyaluronic Acid Immobilized Magnetic Nanoparticles for Active Targeting and Imaging of Macrophages

Medha Kamat;[a] *Kheireddine El-Boubbou;*[a] *David C. Zhu;*[b] *Teri Lansdell;*[a] *Xiaowei Lu;*[a] *Wei Li;*[c] *Xuefei Huang*[a]*

[a]Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA

[b]Departments of Radiology and Psychology, Michigan State University, East Lansing, MI 48824, USA

[c]Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Avenue, Memphis, TN 38163, USA

Corresponding author:

Xuefei Huang

Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA

Tel: (517) 355-9715, ext 329

Fax: (517) 353-1793

Email: xuefei@chemistry.msu.edu

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Figure S1. Thermogravimetric analysis of NH_2-DESPION and HA-DESPION. The additional weight loss upon heating of HA-DESPION compared to NH_2-DESPION was attributed to HA immobilized on the NPs.

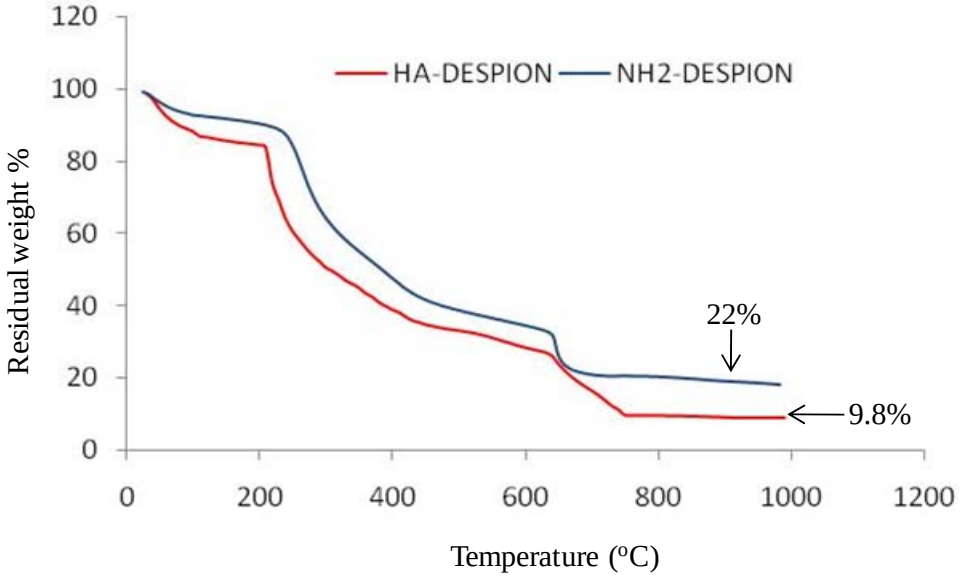
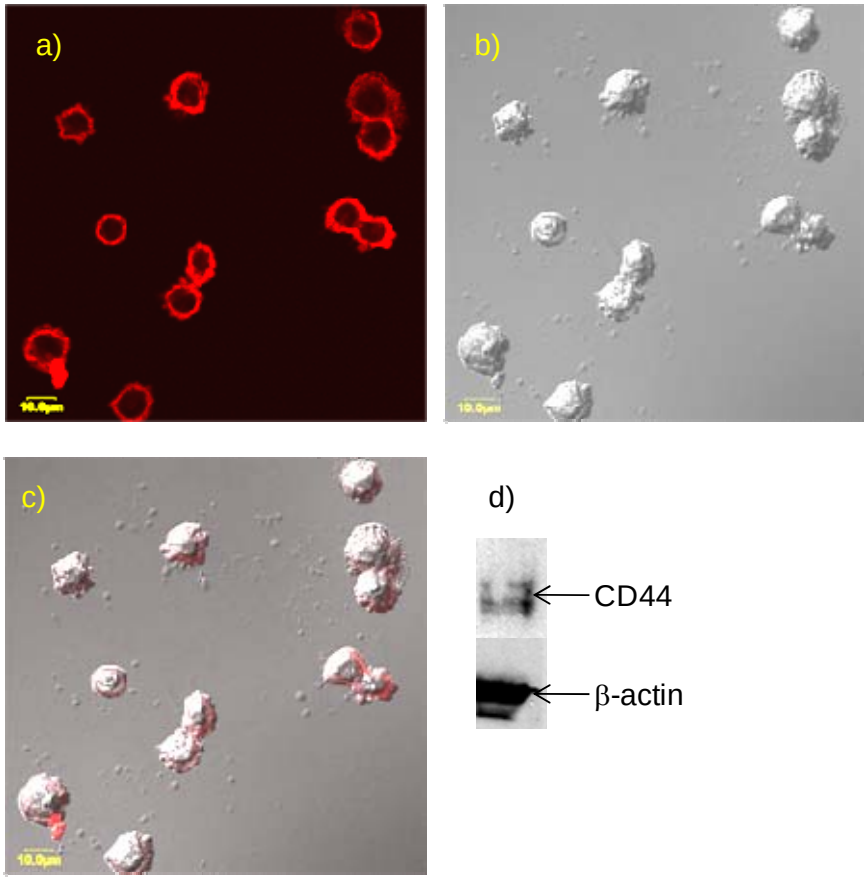


Figure S2. a) Confocal fluorescent microscopy image of THP-1 cells upon incubation with anti-human CD44 mAb followed by Alexafluor 594 labeled anti-IgG secondary antibody; b) Laser image of the cells; and c) Overlay of the fluorescent and laser images. The intense red color in a) suggested the presence of CD44 in THP-1 cells. This was confirmed by d) Western blot of THP-1 cells.



Immunohistostaining for CD44: The cells were inoculated in 4 chambered cover glass (LabTek, Fisher Science) at a cell density of $4x10^5$cells/mL. THP-1 cells were differentiated with PMA(10 μg/mL) to allow adhesion overnight. Next morning the cells were fixed using 10% neutral buffered formalin for 15min, washed with PBS containing 0.05% tween20 (Sigma) (wash buffer) twice for 10 min each. The cells were blocked using PBS containing 10% goat serum and 1% BSA for 2 h at RT. After 2 h, the blocking solution was drained and 300μL of 50μg/mL mouse anti human CD44 mAb (MEM 85, Invitrogen) diluted in PBS containing 1% BSA was added. The samples were then incubated in a moisture chamber at 4^oC for 48 h. For control, the blocking solution was used. Upon completion of incubation period, the supernatants were drained and washed with wash buffer twice for 10 min each. Chambers were dried by decanting and then were treated with 300 μL of 10 μg/mL goat antimouse IgG_{2b} secondary antibody labeled with Alexa Fluor 594 for 1 h, in dark. After an hour, the chambers were

washed buffer twice with 10 min each followed by water, in dark. Samples were imaged using confocal microscopy on Olympus Fluoview 1000 LSM.

Western Blot analysis to determine CD44 expression: The medium was removed from cell culture either by centrifugation or aspiration. The cells were washed with cold PBS twice and then lysed using a mixture of RIPA buffer, protease inhibitor phenyl methyl sulphonyl fluoride (PMSF), and phosphatase inhibitor cocktail. The lysate was centrifuged to remove cell debris. The total protein concentration was determined using Bradford assay (Bio-Rad). The samples together with high-molecular weight prestained standards were electrophoresed on non reducing 10% SDS gel, and then transferred to a PVDF membrane. The membrane was blocked using 4% non-fat milk in TBS-tween (TBST) buffer for 1 h and then treated with mouse anti-human CD44 antibody (156-3C11, Cell signaling) diluted to 1:1000 in 4% non-fat milk in TBST at 4°C overnight followed by goat anti-mouse HRP antibody (Bio-Rad) for 1 h. The blot was sprayed with chemiluminescent HRP detection reagent (Denville Scientific) and developed using autoradiography. β-actin was used as a positive control and was detected using mouse anti-human β-actin peroxidase antibody AC-15 (Sigma-Aldrich,) diluted at 1:40,000 in 4%Milk-TBSTfor 1 h at RT. The blot was developed using aforementioned protocol.

Figure S3. a) Confocal fluorescent microscopy image of EA.hy926 cells upon incubation with anti-human CD44 mAb followed by Alexafluor 594 labeled anti-IgG secondary antibody; b) Laser image of the cells; and c) Overlay of the fluorescent and laser images. The intense red color in a) suggested the presence of CD44 in EA.hy926 cells. This was confirmed by d) Western blot of EA.hy926 cells.

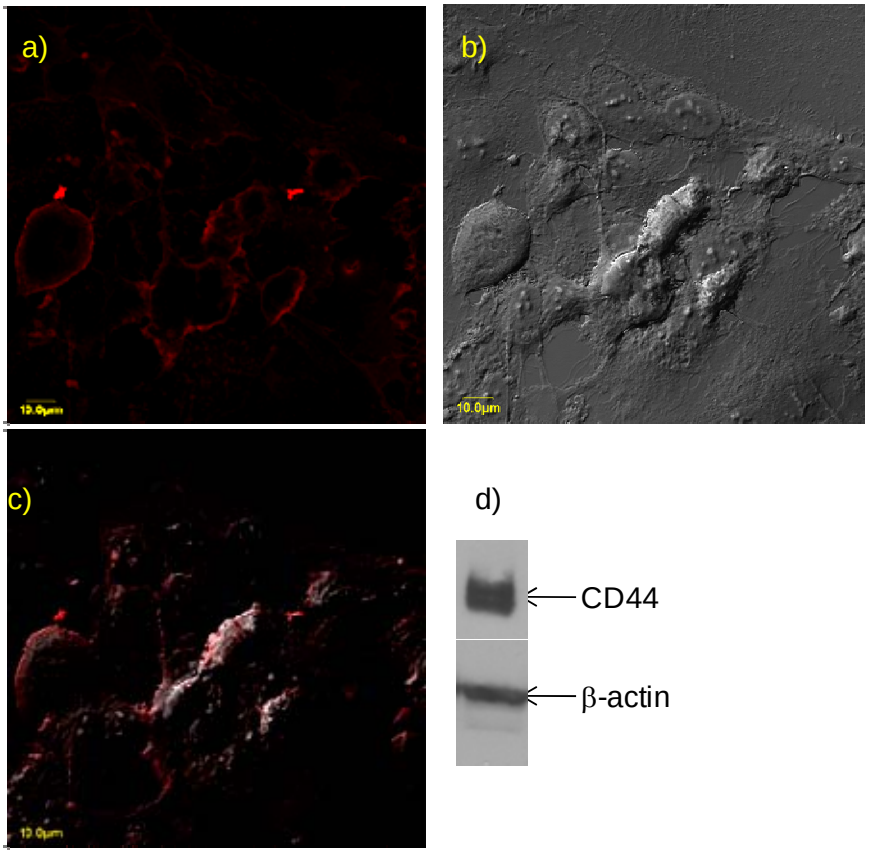


Table S1. Elemental analysis of nanoparticles. The significant increase of % of nitrogen in HA-DESPION confirmed the immobilization of HA on NPs.

Nanoparticles	C (%)	H (%)	N (%)
DESPION	45.09	6.28	0.09
NH_2-DESPION	42.42	6.09	1.02
HA-DESPION	40.09	5.81	3.03

Table S2. Concentration of TNF-α released from THP-1 cells under various conditions as determined from an anti-TNF-α ELISA.

	Treatment method	Amount of TNF-α (pg/mL)
1	Control	0
2	Media only	0
3	PMA (10 ng/mL)	37.5
4	Cholesterol (10 ng/mL)	40.5
5	PMA + LPS (10 ng/mL)	2500

Anti-TNF-α ELISA: The THP-1 cells were treated with endotoxin free media, PMA containing media (10 ng/mL, 16 h), and LPS containing media (10 ng/mL, 2 h), at a density of $5x10^5$ cells/mL. After completion of the treatment, the supernatant was collected, centrifuged to remove cellular debris, and subjected to ELISA. The ELISA was performed according to the manufacturer's instructions (R&D systems) to determine the cytokine concentration.