Materials and Detailed Methods

1. Materials

1-Hydroxybenzotriazole hydrate (HOBT) was purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA), and poly(lactic-co-glycolic acid) (PLGA, lactic acid: glycolic acid = 50:50, with a carboxylic acid terminus) was purchased from Durect Corporation (4.2 kDa) or Lakeshore Biomaterials (10 kDa). Methyl 3,5-diaminobenzoate was purchased from Polysciences, Inc (Warrington, PA). 4-Dimethylaminopyridine (DMAP), quinic acid, acetic anhydride (Ac_2O), anhydrous pyridine, anhydrous dimethylformamide (DMF), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC), dichloromethane (DCM), methanol, acetonitrile (AcN), N,N,N',N'-tetramethylethylenediamine (TEMED), lithium hydroxide (LiOH), collagen type-I, phenol red-free medium 199, L-glutamine, 3,3′,5,5′-Tetramethylbenzidine (TMB), bovine serum albumin (BSA), Amberlite acidic resin, di-tert-butyldicarbonate (Boc_2O), propargyl alcohol, tert-butyl nitrite (t-BuONO), trimethylsilyl azide ($TMSN_3$), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and dichloromethan (DCM) were purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) was purchased from Invitrogen (Eugene, OR, USA). 1,5-bis-4, 8-dihydroxyanthracene-9,10-dione (DRAQ-5) was purchased from Axxora LLC (San Diego, CA, USA). Functionalized polyethylene glycols (carboxyl-PEG-amine, HCl salt, 3.5 kDa and methoxy-PEG-amine, HCl salt, 5kDa) were purchased from JenKem technology (Beijing, China). Recombinant human tumor necrosis factor–α (TNFα) and rat tail collagen-I were purchased from Invitrogen (Grand Island, NY, USA). Anti-Mouse IgG (H+L), HRP conjugate and Luciferase cell culture lysis reagent were purchased from Promega (Madison, WI, USA). Monoclonal anti-human E-selectin/P-selectin (Anti-Eselectin Ab) was purchased from R&D systems (Minneapolis, MN). Human umbilical vein endothelial cells (HUVEC) and Clonetics® EGM® medium were purchased from Lonza (Rockland, ME, USA).

2. Synthesis and analysis

An amine-coupled QA derivative (QA-NH_2, compound **4**) was synthesized in an acetate-protected form (**5**), according to the published method with modifications.[1] The amine group of protected derivative of QA-NH_2 was converted to azide (PQA-Az, compound **5**) for further conjugation to polymeric backbone using click chemistry.[2-4] A bi-functional PEG (compound **6**) was conjugated to compound 5 by click chemistry to produce a protected QA-PEG conjugate. After deprotection, the QA-PEG was conjugated to PLGA via an amide bond yielding a QA-PEG-PLGA polymer (compound **11**). Structures of intermediates and the final product were elucidated using ^{1}H-NMR, ^{13}C-NMR, and electrospray ionization (ESI) mass spectroscopy.

*2.1. Synthesis of (1S,3R,4S,5R)-1,3,4,5-tetraacetoxycyclohexanecarboxylic acid (**2**)*

4-dimethylaminopyridine (DMAP, 20 mg, 0.16 mmol) was added to 961 mg (5 mmol) of quinic acid (**1**) dissolved in 12 mL of acetic anhydride (Ac_2O)-pyridine 1:2 mixture at 5ºC. The reaction was completed in 14 h according to NMR analysis. The reaction mixture was then added to 50 mL of ice water, acidified to pH 3 with 1.2 M HCl (50 mL), and extracted with 70 mL of dichloromethane (DCM) 4 times. The extracts were combined and dried over sodium sulfate (Na_2SO_4), and the solvent was removed by rotary evaporation.

^{1}H-NMR δ ppm: 9.7 (1H, s), 5.523-5.487 (1H, q, J = 3.47 Hz), 5.426-5.342 (1H, m), 5.004-4.959 (1H, dd, J = 3.5 Hz, J = 9.8 Hz), 2.657-2.496 (2H, m), 2.363-2.298 (1H, dd, J = 3.5 Hz, J = 15.8 Hz), 2.08 (3H, s), 2.025 (3H, s), 1.989 (3H, s), 1.935 (3H, s). ^{13}C-NMR δ ppm: 174.393, 70.214, 170.027, 169.983, 169.870, 78.326, 71.400, 67.569, 66.337, 36.503, 31.453, 20.845, 20.791, 20.7178, 20.532.

*2.2. Synthesis of (1R,2S,3R,5S)-5-(3-amino-5-(methoxycarbonyl)phenylcarbamoyl) cyclo hexane -1,2,3,5-tetrayl tetraacetate (**3**)*

N-Hydroxybenzotrizole (HOBT, 431.85 mg, 3.19 mmol) was added to 957.6 mg (2.7 mmol) of compound 2 dissolved in 15 mL of dimethylformamide (DMF) at 0°C under argon. After 5 minutes, 611.6 mg (3.19 mmol) of (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) was added to the reaction mixture, followed by addition of 0.5 mL (4.32 mmol) triethyl amine (TEA). After 1 h stirring, 1.74 g (7.7 mmol) of methyl 3,5-diaminobenzoate dissolved in 5 mL DMF was added under argon using a syringe, and the reaction mixture was warmed up to room temperature. The reaction mixture was stirred for 72 h until the completion of reaction. The product was added to 150 mL ice cold water and extracted 4 times with 300 mL of DCM. The combined organic extracts were washed with 50 mL of 1.5 M HCl solution twice, followed by three washes with 100 mL of brine solution. The organic extract was dried over sodium sulfate, and the solvent was removed with a rotary evaporator. After purification by flash chromatography on silica gel (hexane:ethyl acetate = 3:7), the desired product (**3**) was collected.

Yield (the amount of the recovered product divided by the sum of reactants): 75%. ^{1}H-NMR δ ppm: 9.686 (1H, s), 7.31 (1H, s), 7.162 (1H, s), 6.906 (1H, s), 5.402-5.426 (2H, m), 5.084-5.097 (1H, dd, J = 10.5 Hz, J = 3.6 Hz), 3.780 (3H, s), 2.126 (3H, s), 2.060 (3H, s), 1.955-1.987 (10H, m). ^{13}C-NMR δ: 170.665, 170.589, 170.519, 170.409, 169.195, 167.503, 150.128, 140.219, 131.035, 111.052, 110.886, 110.010, 81.359, 71.978, 68.761, 67.349, 52.771, 37.275, 31.344, 22.373, 21.669, 21.603, 21.399. ESI $(M+H)^{+}$: 510.

*2.3. Synthesis of 3-amino-5-((1S,3R,4S,5R)-1,3,4,5 tetrahydroxycyclo hexanecarbo xamido) benzoic acid (QA-NH_2, **4**)*

To compound 3 (102.2 mg, 0.2 mmol) dissolved in 5 mL dry tetrahydrofuran (THF) was added 0.8 mL of aqueous lithium hydroxide (LiOH). The reaction mixture was stirred for 24 h. Amberlite acidic resin was added to the reaction vessel until the pH

decreased from 10 to 5. The crude product was filtered and purified by reverse column chromatography (water:acetonitrile = 1:2) to yield compound 4.

Yield: 47%. ^{1}H-NMR δ ppm: 7.124 (1H, s), 7.024 (1H, s), 6.809 (1H, s), 5.302-5.326 (2H, m), 5.084-5.097 (1H, m), 2.454-2.644 (4H, m). ESI $(M+H)^{+}$: 327.

*2.4. Synthesis of (1R,2S,3R,5S)-5-((3-azido-5-(methoxycarbonyl) phenyl)carbamoyl) cyclohexane-1,2,3,5-tetrayl tetraacetate (PQA-Az, **5**)*

Compound 3 (100 mg, 0.2 mmol) was dissolved in 2 mL acetonitrile and cooled to 0°C in an ice bath. To this stirred mixture was added tert-butyl nitrite (t-BuONO, 30.5 mg, 35 µL, 0.3 mmol) followed by trimethylsilyl azide ($TMSN_3$, 27.5 mg, 31.5 µL, 0.25 mmol) dropwise. The resulting solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under vacuum, and the crude product was purified by silica gel chromatography (ethyl acetate:Hexane = 2:1).

Yield: 50%. ^{1}H-NMR δ: 7.359-7.692 (2H, m), 7.358 (1H, s), 5.622-5.644 (1H, m), 5.386-5.439 (1H, m), 5.028-5.073 (1H, m), 3.880 (1H, s), 2.860-2.88 (1H, m), 2.554-2.608 (3H, m), 2.173-2.185 (3H, m), 1.951-2.076 (10 H, m). ESI $(M+H)^{+}$: 535.

*2.5. Synthesis of (tert-butoxycarbonylamino)methoxy polyethylene glycol (HOOC-PEG-NHBoc, **7**).*

To HOOC-PEG-NH_2 (3.5 kDa, 500 mg, 140 mmol, compound 6) dissolved in methanol was added di-tert-butyldicarbonate (Boc_2O, 21.21 mg, 36 µL, 0.15 mmol) and TEA (38.2 mg, 53 µL, 0.35 mmol). The reaction continued overnight at room temperature. The crude product was dialyzed against water and lyophilized to yield pure HOOC-PEG-NHBoc. Yield: 95%. ^{1}H-NMR δ ppm: 3.56 (s), 1.45 (s).

*2.6. Synthesis of (tert-butoxycarbonylamino)methoxy, N-(prop-2-yn-1-yl)acetamide polyethylene glycol (propargyl-PEG-NHBoc, **8**).*

Compound 8 was synthesized by a published technique with modification.[5] Briefly, to compound 7 (50 mg, 0.015 mmol) dissolved in dioxane was added HOBT (3.8 mg, 0.03 mmol), EDC (7.5 µL, 0.04 mmol, 6.65 mg) and TEA (12 µL, 9.2 mg, 0.09 mmol). The mixture was stirred under argon for 2 h. Propargyl amine (16 mg, 19 µL, 0.3 mmol) was added to the reaction mixture, and the reaction continued for 12 h. The solvent was evaporated, and the product was redissolved in DMSO, dialyzed against DMSO followed by water exchange and lyophilization.

Yield: 55%. Due to the small number of protons added by conjugation of the propargyl group relative to the number of PEG protons, ^{1}H-NMR spectroscopy could not be used to resolve the spectroscopic difference between the compounds 7 and 8.

*2.7. Synthesis of (tert-butoxycarbonylamino)methoxy-polyethylene glycol-(1R,2S,3R,5S)-5-((3-(4-(11,11-dimethyl-3,9-dioxo-6,10-dioxa-2,8-diazadodecyl)-1H-pyrazol-1-yl)-5-(methoxycarbonyl)phenyl)carbamoyl)cyclohexane-1,2,3,5-tetrayl tetraacetate (**9**).*

To compound 8 (50 mg, 0.015 mmol) and compound 5 (15 mg, 0.03 mmol) dissolved in a 5 mL mixture of water and butanol (2:1) was added a few crystals of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate (6 mg, 0.03 mmol). The reaction vessel was sealed under argon, and the mixture was stirred overnight at room temperature. The reaction mixture was dialyzed against water and lyophilized to yield compound 9.

Yield: 50%. According to NMR quantification based on comparison of protons of the aromatic ring in QA-NH_2 (7-8 ppm) and those of PEG (3.5-3.7 ppm), the ratio of compound 5 conjugated to PEG was estimated to be 50 mol%. ^{1}H-NMR δ ppm: 7.4 (m), 3.56(s), 1.45(s).

*2.8. Synthesis of (amino)-polyethylene glycol-3-(4-((3-(aminomethoxy)propanamido) methyl)-1H-pyrazol-1-yl)-5-((1S,3R,4S,5R)-1,3,4,5-tetrahydroxycyclohexanecarboxamidobenzoic acid (QA-PEG, **10**).*

For deprotection of compound 9, 50 µL trifluoroacetic acid (TFA) was added to compound 9 solution in dioxane. The mixture was stirred for 4 h and neutralized with 1M NaOH. Subsequently, 200 µL LiOH was added to the reaction mixture and stirred overnight. After removing the organic solvent by evaporation, the aqueous solution was dialyzed against water and lyophilized to obtain pure compound 10. Yield: 50%.

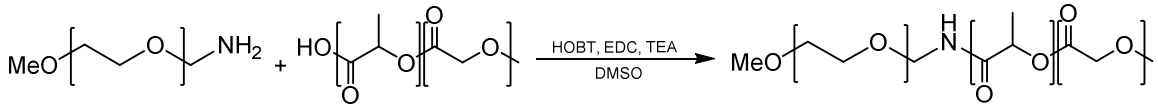
*2.9. Synthesis of 3-(4-((3-(aminomethoxy)propanamido)methyl)-1H-pyrazol-1-yl)-5-((1S,3R,4S,5R)-1,3,4,5-tetrahydroxycyclohexanecarboxamidobenzoic acid)-polyethylene glycol- poly(lactic-co-glycolic) acid (QA-PEG-PLGA, **11**).*

To PLGA (4.2 kDa, 100 mg, 0.024 mmol) in DMSO was added HOBT (5 mg, 0.04 mmol), EDC (8.5 µL, 0.05 mmol) and N,N-diisopropylethylamine (12.5 µL, 0.07 mmol). The mixture was stirred under argon for 2 h. Compound 10 (83 mg, 0.023 mmol) was added to the reaction mixture, and the reaction continued overnight. The product was purified by dialysis in DMSO followed by solvent exchange with water and lyophilization. NPs were prepared with PLGA-PEG-QA by single emulsion technique and purified by multiple washing with water. The purified NPs were analyzed by NMR spectroscopy.

Yield: 30%. According to comparison of methylene protons of PLGA (5.2 ppm) with methylene protons of PEG (3.5-3.7 ppm), the ratio of QA-PEG conjugated to PLGA was 20 mol%. Therefore, the ratio of QA conjugated to PLGA was 10 mol% (the conjugation of QA to PEG, 50 mol% × the conjugation of QA-PEG to PLGA, 20 mol%). ^{1}H-NMR δ ppm: 5.2 (m), 4.8 (m), 3.7 (s), 1.5(s).

*2.10. Synthesis of poly(lactic-co-glycolic) acid-polyethylene glycol conjugate (PEG-PLGA, **12**).*

PLGA (10 kDa, 100 mg, 0.01 mmol) and HOBT (5 mg, 0.04 mmol) were dissolved in 5 mL DMSO under stirring. EDC (8.5 µL, 0.05 mmol) and N,N-diisopropylethylamine (12.5 µL, 0.07 mmol) were sequentially added to the solution and stirred for 1 h to form activated PLGA. Methoxy-PEG-amine (5 kDa, 200 mg, 0.04 mmol) dissolved in 1 mL DMSO was added dropwise to the reaction mixture and stirred overnight. The formed PEG-PLGA was dialyzed against a 75:25 mixture of water and DMSO using a dialysis membrane with MW cutoff of 10,000 Da to remove the unreacted PEG. Finally, DMSO was exchanged with water, and the aqueous solution was freeze-dried. Percent conjugation of PEG to PLGA, quantified by ^{1}H-NMR spectroscopy after nanoparticle formation, was 10-20mol%. Yield: 45%. ^{1}H-NMR δ ppm: 5.2 (m), 4.8 (m), 3.7 (s), 1.5 (s).



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3. References

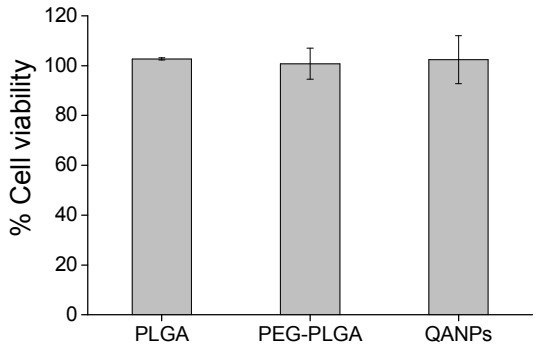
1. Kaila, N.; Somers, W. S.; Thomas, B. E.; Thakker, P.; Janz, K.; DeBernardo, S.; Tam, S.; Moore, W. J.; Yang, R. Y.; Wrona, W.; Bedard, P. W.; Crommie, D.; Keith, J. C.; Tsao, D. H. H.; Alvarez, J. C.; Ni, H.; Marchese, E.; Patton, J. T.; Magnani, J. L.; Camphausen, R. T., Quinic Acid Derivatives as Sialyl Lewis(X)-Mimicking Selectin Inhibitors: Design, Synthesis, and Crystal Structure in Complex with E-Selectin. *J. Med. Chem.* **2005**, 48, 4346-4357.
2. de Graaf, A. J.; Kooijman, M.; Hennink, W. E.; Mastrobattista, E., Nonnatural Amino Acids for Site-Specific Protein Conjugation. *Bioconjugate Chem.* **2009**, 20, 1281-1295.
3. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B., A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed. Engl.* **2002**, 41, 2596-2599.
4. Barral, K.; Moorhouse, A. D.; Moses, J. E., Efficient Conversion of Aromatic Amines into Azides: A One-Pot Synthesis of Triazole Linkages. *Org. Lett.* **2007**, 9, 1809-1811.
5. Marshall, J. A.; Eidam, P. M., A Formal Synthesis of the Callipeltoside Aglycone. *Org. Lett.* **2007**, 10, 93-96.

Supporting Table 1. Particle size and polydispersity index of NPs

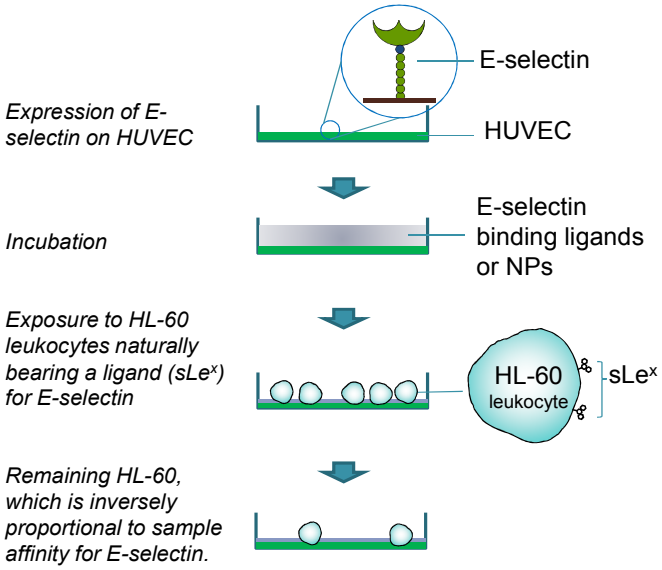
NPs	Size (nm)[a]	Polydispersity index[b]	Zeta potential (mV)
PLGA NPs	179 ± 52	0.15	-17.2 ±1.0
PEG-PLGA NPs	181 ± 27	0.09	-19.5 ± 0.5
QA-PEG-PLGA NPs (QANPs)	192 ± 32	0.21	-19.7 ± 1.2
Ab-PLGA NPs	246 ± 22	0.28	NA
PLGA *NPs	190 ± 27	0.08	
PEG-PLGA *NPs	190 ± 23	0.04	
QA-PEG-PLGA *NPs (QA*NP)	209 ± 38	0.21	
Ab-PLGA *NPs	220 ± 19	0.25	

[a] Data are expressed as averages and standard deviations of 9 independent batches except for Ab-PLGA NPs and Ab-PLGA *NPs (3 independent batches).

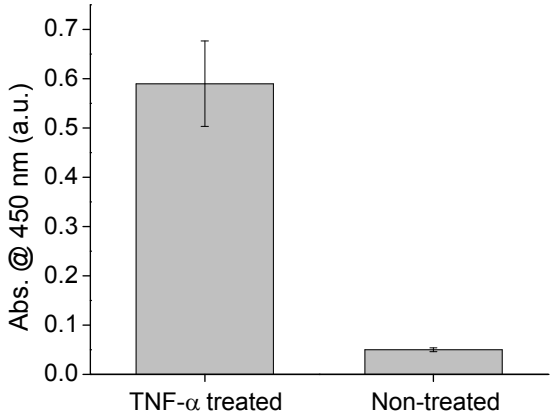
[b] Polydispersity index: an estimate of the width of the particle size distribution, obtained from the cumulants analysis as described in the International Standard on DLS ISO13321 Part 8 (Malvern DLS technical note MRK656-01). Polydispersity index <0.08 is considered monodisperse, and 0.08 to 0.7 is a mid-range polydispersity (Malvern Nano Series and HPPS training manual).



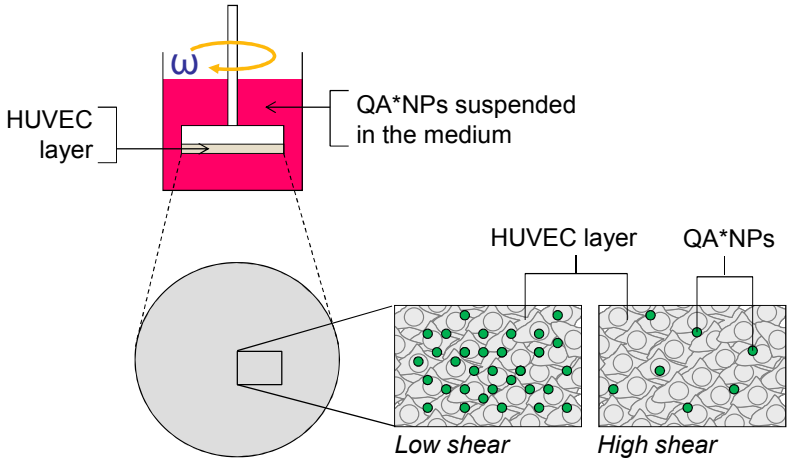
Supporting Fig. 1. Viability of HUVEC exposed to PLGA, PEG-PLGA and QANPs (all at 0.1 mg/mL) for 72 hours. Cytotoxicity of PLGA, PEG-PLGA, and QANPs on HUVEC cells were evaluated based on the MTT assay. HUVEC cells (passage 3) were seeded at a starting density of 10,000 cells per well in a 96-well plate and incubated in 200 μL of Clonetics® EGM® medium to confluence. The culture medium was then replaced with 200 μL of 0.1 mg/mL NPs suspended in fresh medium. After 72 h of incubation, the medium was replaced with 100 μL of fresh medium containing 13% MTT and incubated for 3.5 h. Finally, 100 μL of the solubilization/stop solution containing 20% SDS, 0.02%v/v acetic acid, and 50%v/v DMSO was added to each well, and the absorbance was read at 560 nm by the Tecan microplate reader (Mannedorf, Switzerland). The cell viability was calculated by dividing the absorbance of treated cells by that of untreated cells after subtracting the absorbance of cell-free medium from each. Here, the untreated cells were those provided with no NP suspension but equally handled otherwise, and the cell-free medium was the medium mixed with MTT solution and solubilization/stop solution without cells. Data are expressed as averages with standard deviations of 3 identically and independently prepared samples.



Supporting Fig. 2. Schematic representation of binding-inhibition assay.



Supporting Fig. 3. E-selectin expression on HUVEC activated with 10 ng/mL TNF-α. HUVEC grown overnight in serum-starved medium (SSM) were treated with TNF- α for 4 h, incubated with mouse anti-human E-selectin monoclonal Ab for 1 h at room temperature, and washed 3 times with SSM, followed by the addition of the secondary Ab (anti-mouse IgG-horse radish peroxidase conjugate). Cells were washed after 1h and treated with 200 µL of 3,3′,5,5′-Tetramethylbenzidine (TMB), a substrate for horse radish peroxidase, of which the reaction product was quantified by the measurement of the absorbance at 450 nm. Data represent averages ± standard deviations of 3 identically and independently prepared samples.



Supporting Fig. 4. Schematic representation of rotating disk system (RDS) that creates shear stress on cell layer.