Supporting Information

Leukocyte-Mimicking Stem Cell Delivery via *In situ* Coating of Cells with a Bioactive Hyperbranched Polyglycerol (HPG)

Jae Hyun Jeong, John J. Schmidt, Richie E. Kohman, Andrew T. Zill, Ross J. DeVolder, Cartney E. Smith, Mei-Hsiu Lai, Artem Shkumatov, Tor W. Jensen, Lawrence G. Schook, Steven C. Zimmerman, and Hyunjoon Kong

- Table of Contents –

1. Experimental Procedures

1.1. Synthesis and analysis of VHSPNKK-HPG-g-C_{18}

1.2. Analysis of the association of HPG-g-C_{18} with cells

1.3. Surface Plasmon Resonance (SPR) analysis

1.4. Flow chamber assay

1.5. Flow cytometric assay

2. Supplemental Figures

3. References

1. Experimental Procedures

1.1. *Synthesis and analysis of VHSPNKK-HPG-g-C_{18}*

Hyperbranched polyglycerol (HPG) was synthesized using the slow monomer addition emulsion polymerization method as described by Brooks.[1] The resulting HPG was functionalized with octadecyl chains as follows. To a suspension of KOH (10 equiv. per OH of polyglycerol) in 2 mL of dry dimethyl sulfoxide (DMSO, Aldrich) was added 300 mg of HPG in 1 mL DMSO. This solution was stirred 5 minutes before 60 mg of octadecylbromide (Aldrich) was added. The mixture was stirred for 18 hrs at 60 ºC. The mixture was filtered through celite and the filtrate was washed with hexanes to remove unreacted octadecylbromide. The filtrate was quenched with sodium phosphate buffer and purified by dialysis. The degree of substitution (DS) of octadecyl chains linked to the HPG was determined by ^{1}H-NMR (D_3OD) by comparing the alkyl peak at 1.3 ppm (IntAlkyl) and the polymer backbone peak between 4.0 – 3.4 ppm (IntPoly) as follows.

$$DS_{C18}\,(\%) = \frac{(IntAlkyl/30)}{(IntPoly/5)-(IntAlkyl/30)} \times 100 \qquad (1)$$

For certain experiments to examine association between HPG and a cell, the HPG and HPG-g-C_{18} were labeled with fluorescein by the reaction between lysine residues on the peptide and a fluorescein succinimidyl ester (Invitrogen) (Supplemental Figure S5a).

Next, peptides were attached to HPG-g-C_{18} using an acrylate linker. HPG-g-C_{18} was dissolved in dimethylformamide (DMF, Aldrich) (100 mg/mL) with 6 equivalents (per OH) of triethylamine (Aldrich). The solution was cooled on ice as a 10 % (v/v) solution of acrylchloride (Aldrich) in DMF (5 equiv. per OH) was slowly added. The solution was allowed to stir overnight at room temperature. A DMF solution of peptide NH_2-CG_4VHSPNKKASSKY-

COOH was added to the solution to activate reaction between the thiol groups of peptides and acrylate group of the HPG, at which the molar ratio between two reactants was kept constant at 1:1.[2] After 24 hrs the HPG solution was treated with 20 equivalents of β-mercaptoethanol to quench remaining acrylate groups and dialyzed, sterilized via filtration, and lyophilized. The amount of VHSPNKK peptides attached to the HPG was determined by UV absorbance of the tryptophan residue on the VHSPNKK peptide at 280 nm.

1.2. *Analysis of the association of HPG-g-C_{18} with cells*

In this study, 1.0×10^6 mouse clonally derived mesenchymal stem cells (D1 cells, ATCC) were mixed with 75 μL of HPG or HPG-g-C_{18} solution (2.0 mg/mL) for 10 minutes. Then, the mixture was centrifuged to remove excess HPG not associated with cells. Then, the fluorescent HPG associated with cells was imaged with a fluorescent microscope (Leica DMI 4000).

1.3. *Surface Plasmon Resonance (SPR) analysis*

To evaluate the association between polyglycerol and lipid molecules, a gold sensor chip (GE Healthcare, USA) was modified to present a 11-mercaptoundecanoic acid (MUA, Sigma-Aldrich) monolayer by injecting MUA solution into the flow cell in a Biacore 3000 (GE Healthcare, USA) for 30 minutes at 25 ^{o}C. The carboxylic groups of the MUA monolayer were then activated by flowing 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.05 M *N*-hydroxysuccinimide (NHS, Sigma-Aldrich) solutions through the flow cell for seven minutes. After activation, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE, Sigma-Aldrich) was chemically linked to the MUA layer by flowing DPPE solution until the response unit was saturated. The remaining NHS-ester groups on the MUA surface were blocked by injecting 1.0

M ethanolamine hydrochloride into the flow cell. Then, the top layer was built by injecting a solution of DPPE or recombinant human vascular cell adhesion molecules (VCAM, R&D systems) for five minutes. HPG, HPG-g-C_{18}, VHSPNKK-HPG-g-C_{18}, MSCs associated with the HPG-g-C_{18}, or MSCs associated with VHSPNKK-HPG-g-C_{18} were injected at a flow rate of 5.0 μl/min over the sensor chip. In this study, we used a rat bone marrow derived stromal cells (D1 cell, ATCC) as a model MSC. The kinetic data from SPR sensorgrams were obtained with the assistance of BIA evaluation version 4.1, where a 1:1 Langmuir binding model was applied to quantify the association and dissociation rate constants.[3-5]

1.4. *Flow chamber assay*

Mouse endothelial cells (EC, ATCC) were cultured on collagen-coated glass slides until the cells reach confluence. ECs were exposed to 30 ng/mL of tumor necrosis factor– alpha (TNF-α) for four hours, in order to stimulate the cells to overexpress VCAM.[6] The EC-coated glass slides were then assembled at the bottom of the laminar flow chamber. MSCs mixed with HPG, HPG-g-C_{18}, or VHSPNKK-HPG-g-C_{18} were injected into a custom-made flow chamber at a flow rate of 1.0 ml/min via a 30 mL syringe mounted on a syringe pump. 1.0×10^6 MSCs were injected into the circulation. In this study, we used MSCs derived from porcine adipose tissue, a generous gift from Dr. Mathew B. Wheeler's group in the University of Illinois. The MSCs, which passed over and subsequently adhered to the confluent, VCAM-overexpressing ECs in the flow chamber, were imaged using an inverted microscope (Leica DMI 4000). The focal plane was adjusted to capture the best possible MSC images. Finally, the number of MSCs anchored to the target inflamed endothelium during a given time period was counted using a hematocytometer.

1.5. *Flow cytometric assay*

Porcine adipose derived cells were assayed for expression of CD44, CD45, and CD90 as representative MSC markers (CD44+/CD90+/CD45-). Cells were trypsinized and rinsed in PBS containing 5 % FBS prior to staining. Cells were stained with phycoerythrin (PE) conjugated anti-CD90 and fluorescein conjugated anti-CD44 (BD Bioscience; San Jose, CA) antibodies or with anti-CD45 (AbD serotec) followed by PE conjugated anti-mouse IgG (Santa Cruz Biotechnology) antibodies. Cells were rinsed 3 times with PBS + 5 % FBS prior to analyzing on an iCyt Reflection flow sytometer (Sony Biotechnology).

2. Supplemental Figures

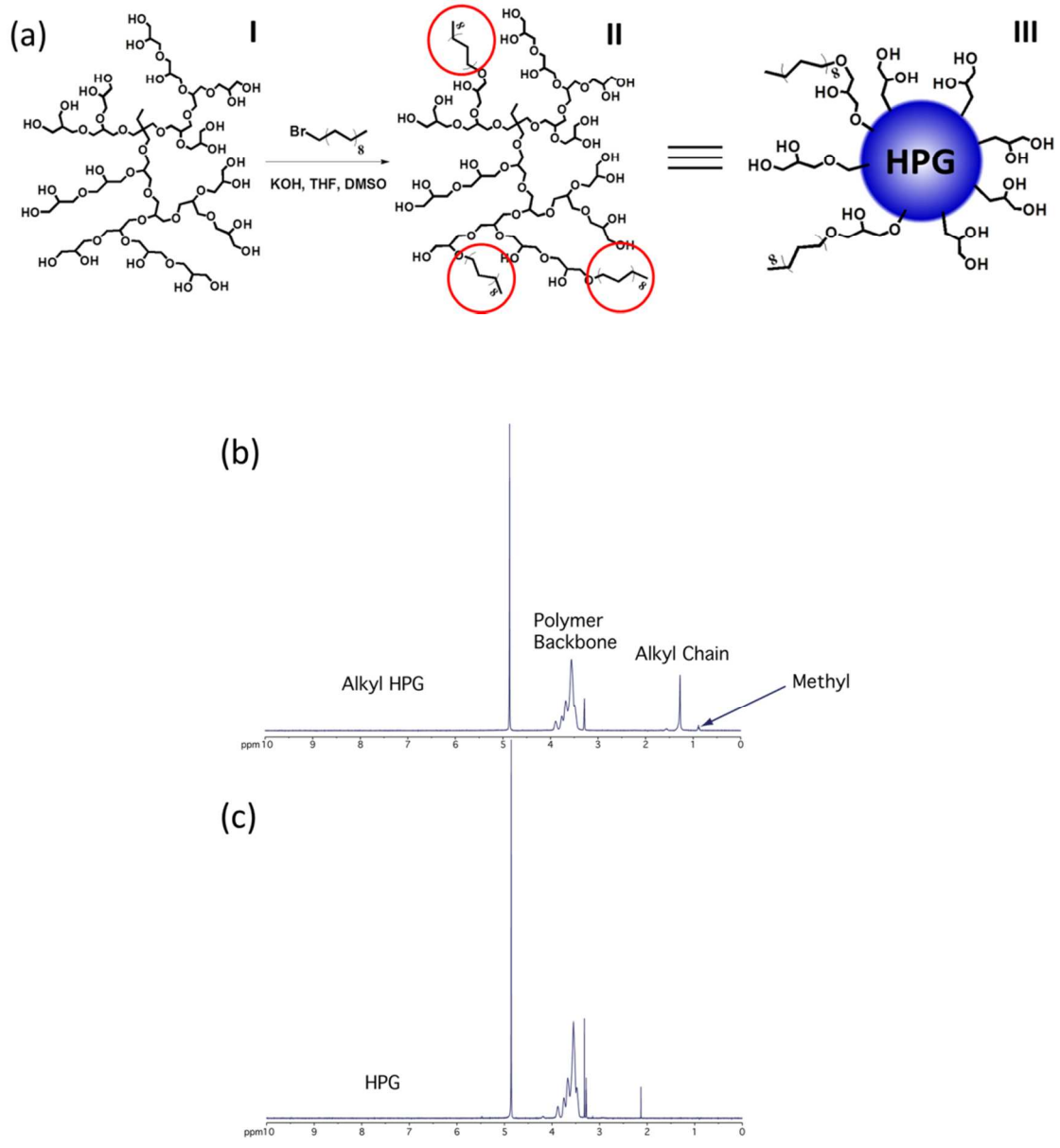

Figure S1. (a) Chemical reaction scheme to prepare polyglycerol-g-C_{18}. ^{1}H NMR analysis of (b) HPG-g-C_{18} and (c) polyglycerol (HPG). In the spectra, every characterization peak was assigned according to the polymer chemical structure.

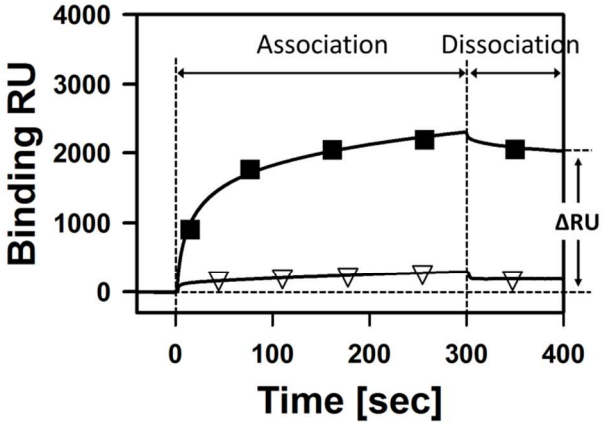

Figure S2. Analysis of polyglycerol binding to lipid bilayers and a mesenchymal stem cell (MSC). SPR response curves for binding of HPG (▽) and HPG-g-C_{18} (■) with the DPPE lipid bilayer built on the SPR chip.

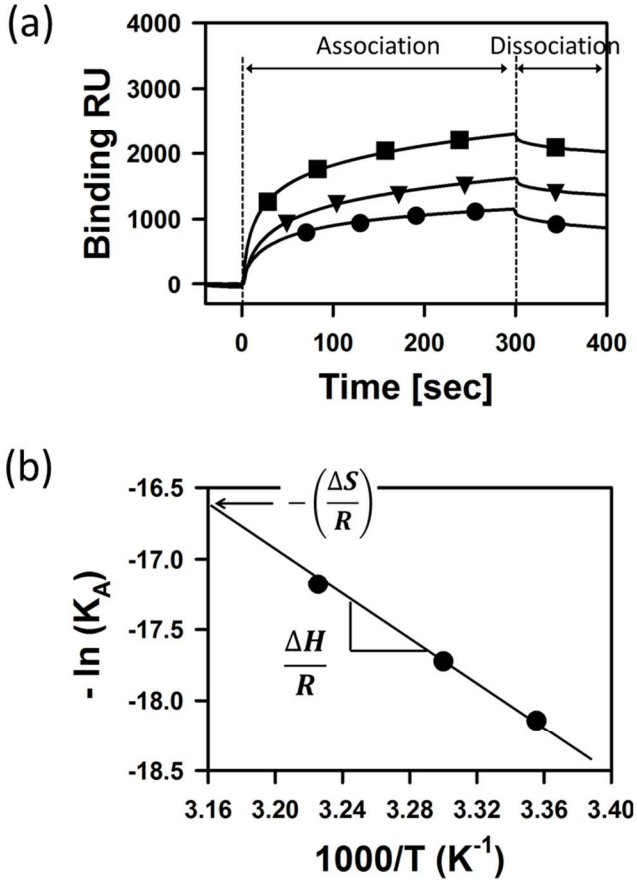

Figure S3. (a) SPR response curves of the HPG-g-C_{18}'s association of and dissociation from the DPPE lipid bilayer at varied temperatures. Temperatures were examined at 25 (■) to 31 (▼) and 37 °C (●). (b) Inverse linear dependency between -ln (K_A) and 1/T for the HPG-g-C_{18}.

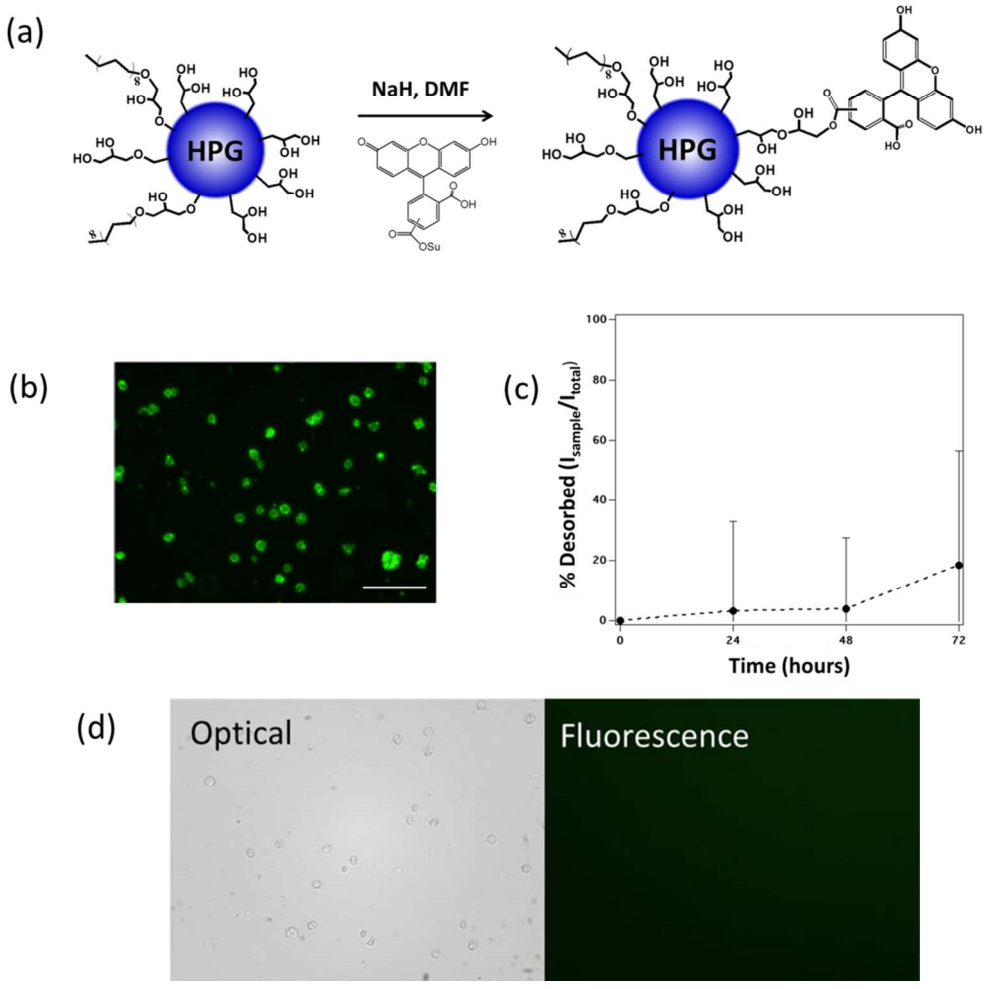

Figure S4. (a) Synthesis of fluorescein-conjugated HPG-g-C_{18} by the reaction between hydroxyl groups on the HPG and fluorescein succinimidyl ester. (b) Fluorescence image of the MSCs associated with fluorescein-conjugated polyglycerol-g-C_{18}. A scale bar represents 100 μm. (c) The fluorescence intensity on the cell surface minimally changed over 72 hours indicating a long residence time for the polyglycerol-g-C_{18} attached to the cell membrane. The percentage of fluorescence intensity was calculated by I_{sample}/I_{total} after background subtraction. (d) Optical and fluorescence image of the MSCs mixed with fluorescein-conjugated HPG. Minimal fluorescence from MSCs confirms minimal association of HPG with cells.

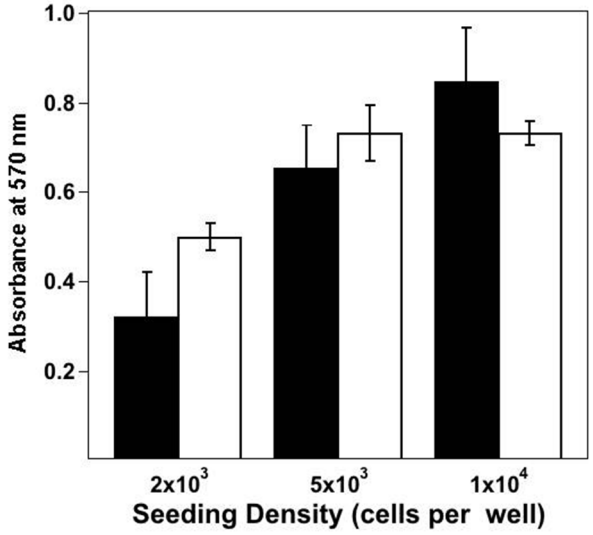

Figure S5. MTT assay to evaluate metabolic activity of MSCs associated with HPG-g-C_{18} (open bar) and unmodified MSCs (filled bar). In this assay, cells were incubated in fresh media containing the MTT reagent for four hours. Metabolically active, viable cells reduce the MTT reagent to a measurable colorimetric formazan dye, and the UV absorbance values of the formazan dye are therefore proportional to the cell density. Next, an MTT detergent was added to the cell culture media, followed by a four-hour incubation at room temperature. The absorbance values of the extracted formazan dye, at 570 nm, were measured using a spectrophotometer (Synergy HT, BioTek). The MTT assay was performed three days after seeding cells on a cell culture flask. The values and error bars of each bar represent average values and standard deviations of three replicates, respectively. For each seeding density, there was no statistical significance between the two conditions.

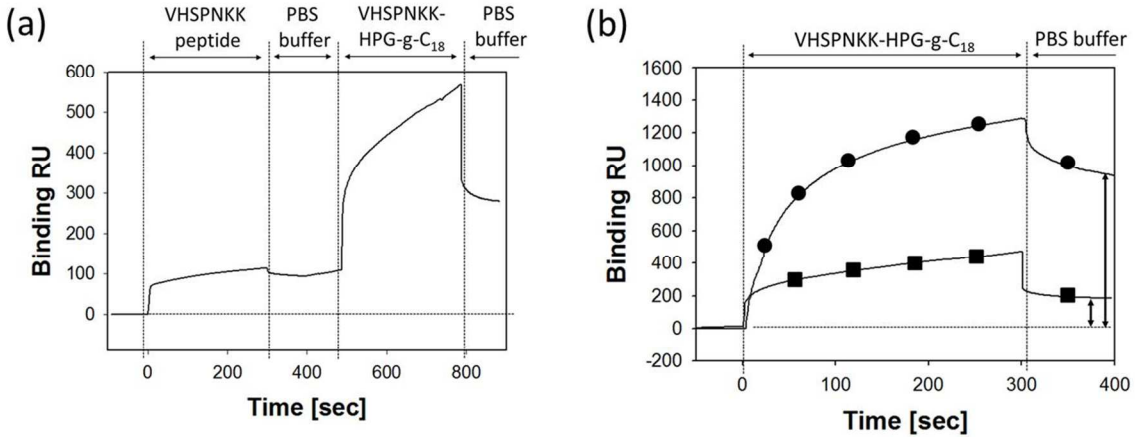

Figure S6. (a) SPR response curve for binding of the free VHSPNKK peptide to the VCAM-coated substrate, followed by injection of the VHSPNKK-HPG-g-C_{18}. (b) The response unit change (ΔRU) of the VHSPNKK-HPG-g-C_{18} was significantly decreased when the VCAM-coated substrate was pre-exposed to the free VHSPNKK peptide. In (b), -■- represents the SPR response curve of VHSPNKK-HPG-g-C_{18}'s association with the SPR-coated substrate pre-exposed to the free VHSPNKK peptide. -●- represents the SPR response curve of VHSPNKK-HPG-g-C_{18}'s association with the SPR-coated substrate in the absence of the free VHSPNKK peptide. 5.0 μM free VHSPNKK peptide and 5.0 μM VHSPNKK-HPG-g-C_{18} solutions were separately injected over the sensor chip at a flow rate of 5.0 μl/min.

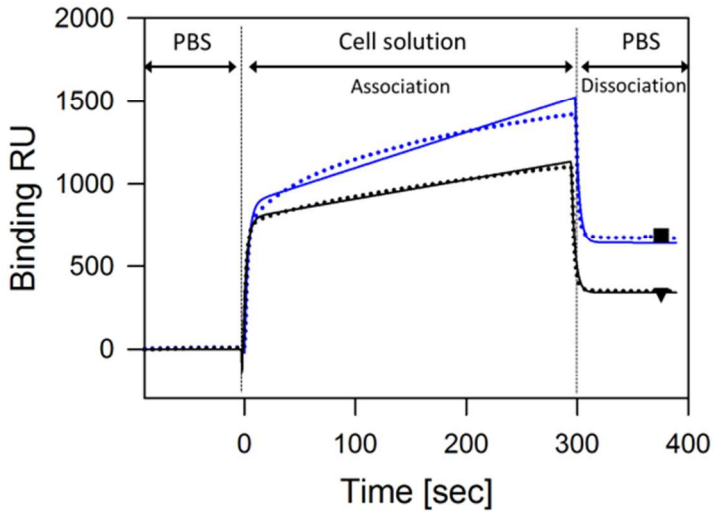

Figure S7. SPR response curves for the binding of modified cells to VCAM coated substrate. The cell solution was injected at a flow rate of 5.0 µl/min over the sensor chip. -▼- represents unmodified MSC cells, and -■- represents modified MSC cells with VHSPNKK-HPG-g-C_{18}, respectively. The dotted lines (··) show binding RU obtained. The solid lines (-) represent global fits to 1:1 Langmuir model (A + B = AB). The scores of X^2 (Chi2) are < 10, indicating that the model used adequately describes the observed binding.

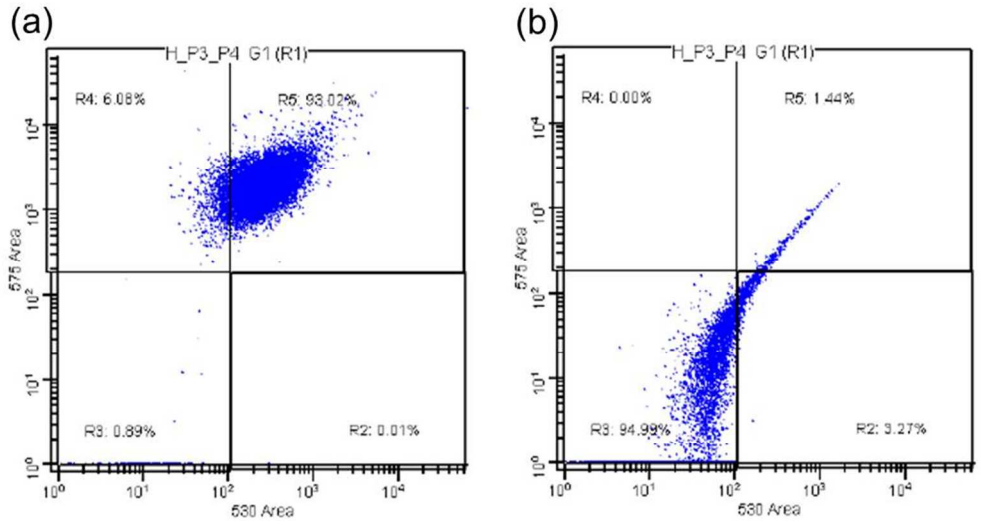

Figure S8. Flow cytometric analysis of porcine adipose-derived MSCs. (a) 99 % of the cells expressed CD44, identified with fluorescent emission at 575 nm, and 93 % of cells expressed CD90, identified with fluorescent emission at 530 nm. (b) Only 0.7 % of the cells expressed CD45, identified with fluorescent emission at 530 nm.

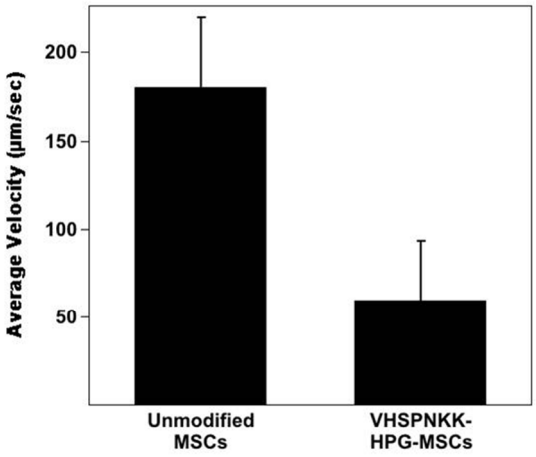

Figure S9. Velocity of MSCs on the inflamed endothelium. All rolling MSCs associated with VHSPNKK-HPG-g-C_{18} were immobilized within 2.4 seconds or less.

3. References

(1) R.K. Kainthan, D.E. Brooks, *Biomaterials* **2007**, *28*, 4779-4787.

(2) M.H.M. Oudshoorn, R. Rissmann, J.A. Bouwstra, W.E. Hennink, *Biomaterials* **2006**, *27*, 5471-5479.

(3) R. Karlsson, A. Michaelsson, L. Mattsson, *J. Immunol. Methods* **1991**, *145*, 229-240.

(4) K. Miyamoto, N. Kodera, H. Umekawa, Y. Furuichi, M. Tokita, T. Komai, Int. *J. Biol. Macromol.* **2002**, *30*, 205-212.

(5) J.H. Jeong, B.Y. Kim, S.J. Lee, J.D. Kim, *Chem. Phys. Lett.* **2006**, *421*, 373-377.

(6) S. Devaraj, B. Davis, S.I. Simon, I. Jialal, *Am. J. Physiol-Heart C.* **2006**, *291*, H1170-H1176.