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

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Abstract

Since stem cells emerged as a new generation of medicine, there are increasing efforts to deliver the stem cells to a target tissue via intravascular injection. However, the therapeutic stem cells lack a capacity to detect and adhere to the target tissue. Therefore, this study presents synthesis of a bioactive hyper-branched polyglycerol (HPG) which can non-invasively associate with stem cells and further guide them to target sites, such as inflamed endothelium. The overall process is analogous to the way in which leukocytes are mobilized to the injured endothelium.

Stem and progenitor cells are being extensively studied as a new generation of medical therapy because of their ability to sustainably produce therapeutic proteins and recreate new tissues.[1–5] One popular cell transplantation strategy is to inject cells into the circulatory system. However, this approach often encounters difficulties in recruiting transplanted cells to the target tissue because therapeutic cells, such as mesenchymal stem cells, lack the capability to bind with target tissue. According to fundamental studies, leukocytes are mobilized to injured or pathologic tissues because they express transmembrane receptors that can specifically bind with proteins overexpressed by the tissue. To mimic the function of leukocytes, efforts are increasingly made to modify the therapeutic cell surface with

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Author Contributions

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Supporting Information

Experimental materials, instruments and procedures; synthesis and characterization of VHSPNKK-HPG-g-C_{18}; analysis of the association of HPG-g-C_{18} with cells; surface plasmon resonance (SPR) analysis, and flow chamber assay; Supplemental videos for the adhesion of modified MSCs to an inflamed endothelium. This material is available free of charge via the Internet at http://pubs.acs.org.

peptides or antibodies that can associate with proteins overexpressed in target tissues.[6–7] For example, the cell surface was first modified with protein linkers such as protein G using activated esters or thiol maleimides, and subsequently exposed to the intercellular adhesion molecules (ICAM) antibodies or E-selectin binding peptides;[8–11] however, such sequential and chemical modification of the cell surface may negatively impact cellular viability and therapeutic activities. Such concern was not well addressed in these past studies.

Therefore, this study presents a nano-sized cell carrier that can non-invasively modify cell surfaces with vasculature binding peptides (VBPs) via *in situ* self-assembly with cells, and regular cellular anchorage to target tissues (Scheme 1). In this study, we hypothesized that hyperbranched polyglycerol (HPG) covalently modified with octadecyl chains and VBPs would bind with cell membranes through hydrophobic chain insertion and display a controlled number of VBPs on the cell surface (I – III in Scheme 1). Additionally, the number of VBP-conjugated HPG on the cell membrane would control the adhesion of transplanted cells to a target tissue (IV in Scheme 1). The HPG possesses a compact, globular architecture which has a restricted conformation, minimizing intermolecular entanglements often encountered with linear polymers.[12–18] Such a molecular architecture is more advantageous for presenting a larger number of octadecyl chains and VBPs than linear polymers.[19]

Additionally, the HPG consisting of polyether-polyol units is highly water-soluble, and minimally stimulates host responses (i.e., high biocompatibility).[20–22] The number of octadecyl chains linked to the HPG would tune the binding affinity between the HPG and cells. In this study, an oligopeptide containing the VHSPNKK sequence was used as a model VBP, because it is known to bind with vascular endothelial adhesion molecule (VCAM) overexpressed by inflamed blood vessels.[23, 26] We evaluated the association and dissociation kinetics of the bio-functionalized HPG on the VCAM-coated substrate, using surface plasmon resonance (SPR) spectroscopy. Further, we demonstrated using an *in vitro* circulation system that the resulting bio-functionalized HPG associates with mesenchymal stem cells (MSCs) and guides them to inflammatory tissue.

First, to develop a hyperbranched polyglycerol (HPG) capable of binding cell surfaces, HPG-g-C_{18} was prepared by a treating a 150 kDa HPG with octadecyl bromide (C_{18}-Br) and base (Figure S1a and SI). ^{1}H NMR analysis indicated that the average of seven C_{18} chains was linked per HPG (Figure S1b; see Eq. (1) in SI). The resulting HPG-g-C_{18} remained soluble in water and could be readily dissolved into cell suspensions. The capability of the HPG-g-C_{18} to associate with cells was evaluated by measuring an association rate constant (k_a), a dissociation rate constant (k_d), and the number of HPG-g-C_{18} adhered to a lipid bilayer simulating a cell membrane, using surface plasmon resonance (SPR) spectroscopy.[24–25] Both k_a and k_d were quantified by fitting SPR curves to 1:1 Langmuir model using Eq. (1),

$$\frac{dRU}{dt} = k_a[A](RU_{max} - RU) - k_d RU \quad (1)$$

where RU_{max} is the maximal response capacity (RU) when the target surface is saturated with HPG-g-C_{18}, and [A] is the concentration of the HPG-g-C_{18}. As expected, the HPG-g-C_{18} exhibited a higher ΔRU and k_a, and a smaller k_d than the unmodified HPG (Table 1, Figure S2a). Additionally, HPG-g-C_{18} conjugated with fluorescein displayed a limited increase of ΔRU, compared to non-fluorescent HPG-g-C_{18}.

Additionally, the underlying mechanism by which the C_{18} chains improve the association of HPG with the cell membrane was examined by quantifying the enthalpy (ΔH), entropy

(ΔS), and Gibbs free energy (ΔG).[27] The measured K_A of HPG-g-C_{18} decreased with increasing temperature, and a plot of –ln(K_A) vs. 1/T fits a linear regression (Table 2, Figure S3). The ΔG calculated from the linear regression using van't Hoff equation, Eq. (2),

$$\Delta G = -RT\ln(K_A) \quad (2)$$

was approximately −11 kcal/mol. The ΔH and ΔS values were determined from the slope and abscissa, respectively, of the linear regression curve between ln (K_A) and 1/T, using Eq. (3).

$$-\ln(K_A) = \left(\frac{\Delta H}{R}\right)\left(\frac{1}{T}\right) - \frac{\Delta S}{R} \quad (3)$$

The ΔH and ΔS values were −14.2 kcal/mol and 11.7 cal/mol·K, respectively. These results indicate that the thermodynamically favorable association between HPG-g-C_{18} and lipid membrane is driven by the negative enthalpy.

Furthermore, mixing the HPG-g-C_{18} with CD44/CD90-positive and CD45-negative mesenchymal stem cells (MSCs) resulted in the coating of MSCs with HPGs after a ten-minute period. Thus, fluorescein-conjugated HPG-g-C_{18} was observed to stain the cell surface, whereas, MSCs mixed with the unmodified polyglycerol exhibited minimal fluorescence (Figure S4). The fluorescence intensity on the cell surface minimally changed over 72 hours indicating a long residence time for the HPG-g-C_{18} attached to the cell membrane (Figure S4). The MSCs associated with HPG-g-C_{18} displayed minimal difference of metabolic activity, as compared to uncoated cells (Figure S5).

Next, to target damaged tissue, the HPG-g-C_{18} was modified with a VCAM-binding peptide containing the VHSPNKK sequence, termed VHSPNKK peptide. For the peptide conjugation, certain hydroxyl groups of HPG-g-C_{18} were modified to present acrylate groups (Figure 1a). These Michael-acceptor groups could be conjugated to the thiol group of the VHSPNKK peptide upon treatment with base. Using the UV absorbance of the tryptophan residue of the VHSPNKK peptide, it was found that the HPG-g-C_{18} contained on average ten peptide groups per alkyl groups.

The capability of the VHSPNKK-HPG-g-C_{18} to bind with a VCAM-coated substrate was evaluated using SPR. As expected, the unmodified HPG-g-C_{18} displayed a minimal adhesion to the substrate, as marked with almost zero value of the response unit change (ΔRU) (Figure 1b). In contrast, the VHSPNKK-HPG-g-C_{18} exhibited an affinity constant six-fold larger than the peptide-free HPG-g-C_{18}. Accordingly, the number of VHSPNKK-HPG-g-C_{18} bound to the VCAM-coated substrate, represented by the response unit change, increased in proportion to the number of HPG added to the SPR unit. Ultimately, the number of HPG molecules bound to the VCAM substrate was 10 times larger than the peptide-free HPG-g-C_{18} after a 300 sec time period (Figure 1c). However, the VHSPNKK-HPG-g-C_{18} exhibited a minimal adhesion to a substrate pre-exposed to free VSHPNKK peptide (Figure S6). Taken together, these results indicate that the VHSPNKK peptide plays a critical role in the targeted adhesion of HPG to VCAM-coated substrate.

Finally, we examined the ability of VHSPNKK-HPG-g-C_{18} to guide mesenchymal stem cells (MSCs) to a target VCAM-coated substrate using SPR (Figure 2a). Interestingly, MSCs coated with VHSPNKK-HPG-g-C_{18} displayed a 1.5-fold increase in the association rate constant (k_a) and 70 % decrease in the dissociation rate constant (k_d), as compared to uncoated MSCs (Figure 2b, Figure S7). Additionally, the affinity constant (K_A) of MSCs

associated with the VHSPNKK-HPG-g-C_{18} was three times larger than the K_A value of uncoated MSCs.

Furthermore, we examined the ability of VHSPNKK-HPG-g-C_{18} to direct the adhesion of CD44/CD90-positive and CD45-negative adipose-derived MSCs to an inflamed endothelium using a blood vessel-mimicking circulation system (Figure S8, Figure 3a). The inflamed endothelium was prepared by exposing an endothelial cell sheet to tumor necrosis factor (TNF)-α, which induced endothelial cells to overexpress VCAM.[28] A flow rate of MSC-suspending fluid was kept constant at 1.0 ml/min, in order to keep shear stress on endothelium to be comparable to coronary artery wall shear stress. Both uncoated MSCs and MSCs associated with peptide-free HPG-g-C_{18} displayed minimal cell adhesion to the inflamed cells (Figure 3b). In contrast, MSCs coated with VHSPNKK-HPG-g-C_{18} displayed significantly smaller rolling velocities compared to uncoated cells, and actively adhered to the inflamed endothelium (Figure 3b, Figure S9). Sphere-shaped MSCs with a 10 μm diameter were readily distinguished from any debris in the cell culture media and endothelial cells fully spread on a substrate. Ultimately, there was a two-fold increase in the number of MSCs adhered to the endothelium when using VSPNKK-HPG-g-C_{18} (Figure 3c). In addition, the MSCs associated with VHSPNKK-HPG-g-C_{18} molecules displayed limited adhesion to the endothelial cells not exposed to TNF-α. These results support the finding that the peptide-conjugated HPG is capable of delivering cells exclusively to the target inflammatory tissue.

In summary, this study demonstrates a novel cell-guidance molecule which can associate with cells in a minimally invasive manner and further guide them to target sites, such as inflamed endothelium. The HPG modified with octadecyl chains associated with MSCs in a thermodynamically favorable manner. Subsequent modification of the HPG-g-C_{18} with VHSPNKK peptides provided a HPG molecule with the ability to bind with VCAM-coated substrate. Ultimately, coating stem cell surfaces with the VHSPNKK-HPG-g-C_{18} significantly enhanced the cellular affinity for the VCAM protein, and therefore successfully directed the anchorage of cells to the target inflamed endothelium. The overall process is analogous to the way in which leukocytes are mobilized to the injured endothelium.[29] Additionally, this approach to functionalize cell membrane with VHSPNKK-HPG-g-C_{18} via self-assembly should be superior to prior, multi-step chemical modifications of cell membranes.

We envisage that the strategy described herein, using a HPG carrier to bind cells and display targeting groups for delivery, would be highly useful for guiding a wide array of therapeutic cells to target tissues following intravascular injection. Such enhanced cell adhesion and subsequent increased cell quantities at target tissues would greatly improve the outcome of cell therapies. In addition, this functionalized HPG may prove useful for guiding various nano- or micro-sized drug carriers to target tissues. These possibilities are the focus of our current studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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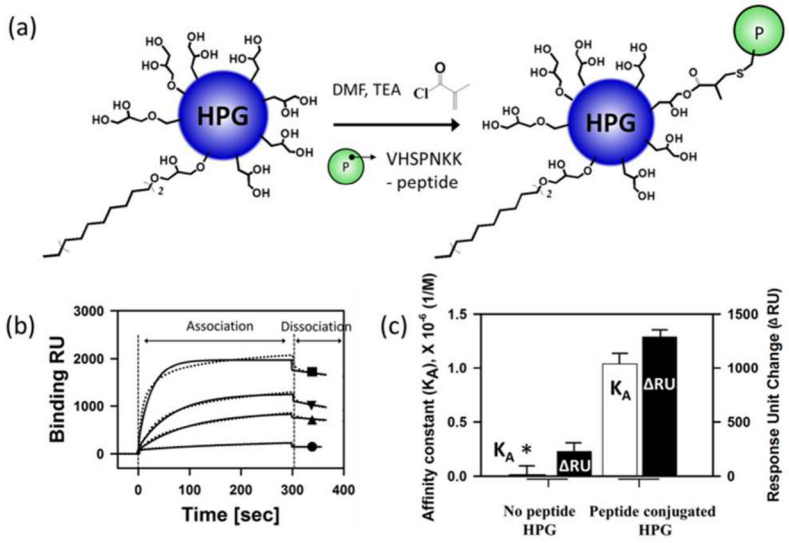


Figure 1.

Bioconjugation of HPG-g-C_{18} with VHSPNKK peptides and SPR analysis of the binding VHSPNKK-HPG-g-C_{18} with VCAM. (a) Chemical reaction scheme to conjugate HPG-g-C_{18} with VHSPNKK peptides. (b) SPR response curves of the VHSPNKK-HPG-g-C_{18}'s association of and dissociation with the VCAM-coated substrate. (c) SPR analysis of the affinity constant (K_A) and the response unit change (ΔRU) of VHSPNKK-HPG-g-C_{18} for the VCAM-coated substrate. In (b), -●- represents peptide-free polyglycerol with 5.0 μM. The concentration of VHSPNKK-HPG-g-C_{18} incorporated into the flow of SPR unit was varied from 1.2 (-▲-), 5.0 (-▼-), and 10 μM (-■-). The dotted lines (··) show binding RU obtained. The solid lines (-) represent global fits to 1:1 Lang-muir model (A + B = AB). The scores of X^2 (Chi2) in 1.2 and 5.0 μM are <10, indicating that the model used adequately describes the observed binding.

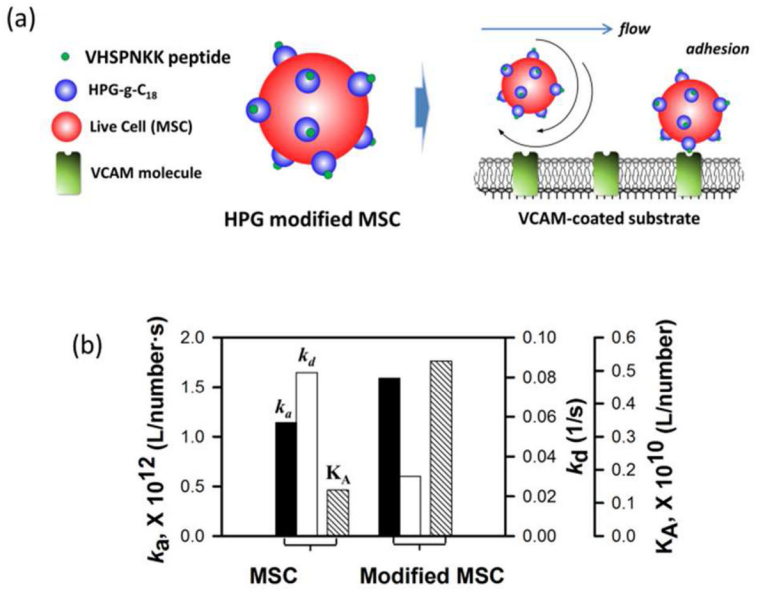


Figure 2.

In vitro evaluation of the function of VHSPNKK-HPG-g-C_{18} to regulate adhesion of MSC to an inflamed endothelium. (a) Schematic of the SPR analysis to characterize adhesion of MSC to a target VCAM-coated substrate. (b) Association rate constant (k_a), dissociation rate constant (k_d), and affinity constant (K_A) of uncoated MSCs and MSCs associated with VHSPNKK-HPG-g-C_{18}, characterized by SPR response curves.

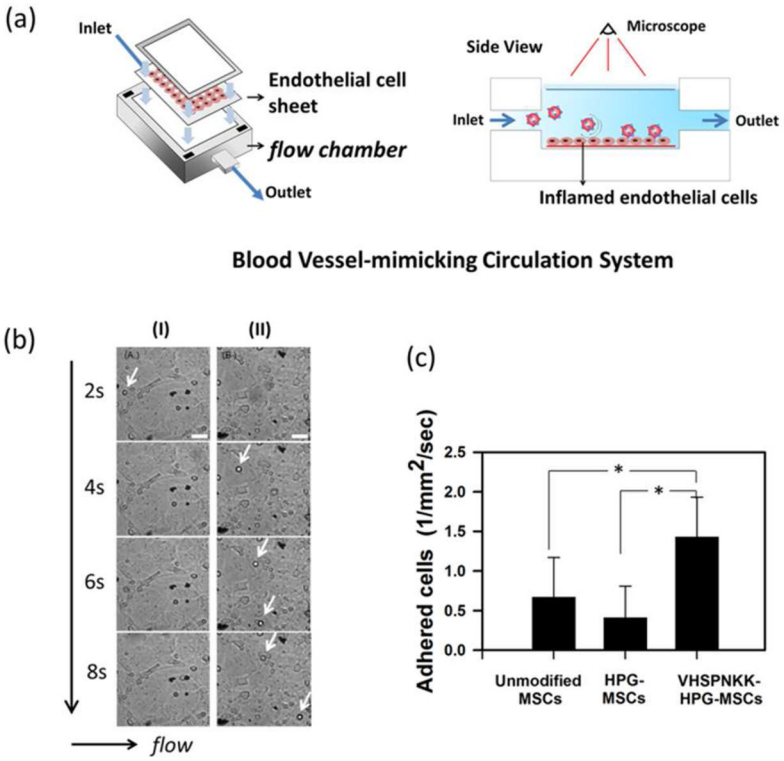
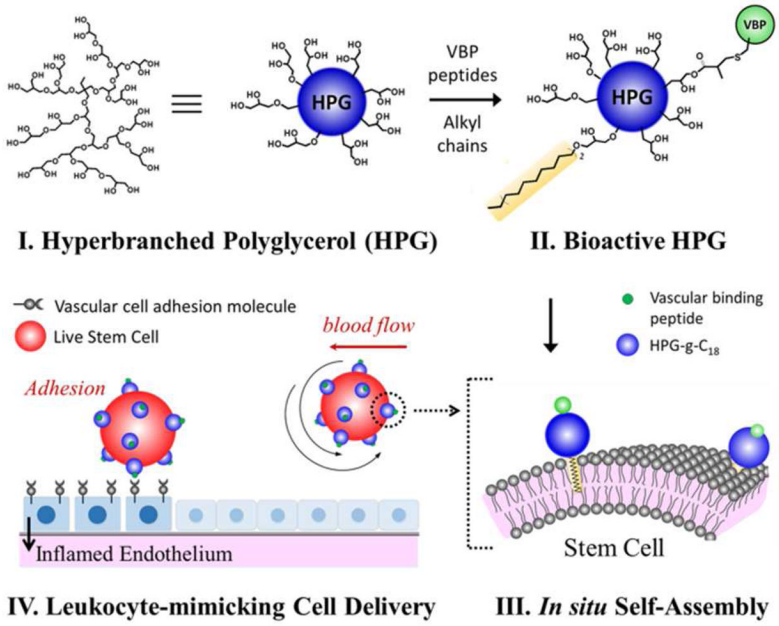


Figure 3.

In vitro evaluation of the function of VHSPNKK-HPG-g-C_{18} to regulate adhesion of MSC to an inflamed endothelium. (a) Schematic of the MSC delivery to a target inflamed endothelium using an in vitro circulation system. (b) Images of the inflamed endothelial cells exposed to the flow of uncoated MSCs (I) and MSCs associated with VHSPNKK-HPG-g-C_{18} (II). Arrows indicate the MSCs anchored to the endothelial cells. The scale bar represents 50 μm. (c) Quantification of the number of MSCs adhered to the target inflamed endothelial cells during a given time period. * represents a statistical significance of the difference between conditions (* $P < 0.05$).



Scheme 1.

A bioactive hyperbranched polyglycerol (HPG) covalently modified with octadecyl chains and vasculature binding peptides (VBPs) was utilized as a novel cell-guidance molecule which can associate with stem cells and further guide them to target defective vasculature.

Table 1

SPR response unit changes (ΔRU) and calculated surface density of unmodified HPG and HPG-g-C_{18} towards the lipid bilayer.

Carriers	Response unit change (ΔRU)[a]	Surface density (ng/mm^2)[b]	Surface HPG number density (number/mm^2)[c]
HPG	180	0.18	7.2×10^8
HPG-g-C_{18}	1900	1.9	7.5×10^9

[a] Response unit change (ΔRU) from the binding of HPG on the lipid bilayer.

[b] Calculated from the response unit (RU).

[c] Calculated from the RU and Mw of HPG (150 kDa) on the lipid bilayer.

Table 2

Quantified analysis of the effect of temperature on the association rate constant (k_a), dissociation rate constant (k_d), affinity constant (K_A), and changes of enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG) of HPG-g-C_{18} towards the lipid bi-layer.

Temp.	Conc. (mg/ml)	Response unit change (ΔRU)	$k_d \times 10^{-4}$ (1/s)	$k_a \times 10^4$ (1/M·s)	$K_A \times 10^7$ (1/M)
298 K	0.05	1900	3.98	3.03	7.6
303 K	0.05	1300	4.73	2.37	5.0
310 K	0.05	780	9.79	2.92	2.9