Chemical Engineering of Mesenchymal Stem Cells to Induce a Cell Rolling Response

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Covalently conjugated sialyl Lewis X (SLeX) on the mesenchymal stem cell (MSC) surface through a biotin−streptavidin bridge imparts leukocyte-like rolling characteristics without altering the cell phenotype and the multilineage differentiation potential. We demonstrate that the conjugation of SLeX on the MSC surface is stable, versatile, and induces a robust rolling response on P-selectin coated substrates. These results indicate the potential to increase the targeting efficiency of any cell type to specific tissue.

Cell therapy including platelet transfusions, bone marrow cell infusion, and islet cell transplantation have had a profound impact on improving patient quality of life and extending patient survival overall, and have demonstrated an impressive safety record (*1*). Culture expanded mesenchymal stem cells (MSCs), also referred to as connective tissue progenitor cells, have been approved for a limited number of applications (*2*) and have recently entered preclinical and clinical trials for treatment of numerous ailments including heart disease (*3*−*5*), brain and spinal cord injury (*6*), cartilage and bone injury (*7, 8*), Crohn's disease (*9*), and graft versus host disease during bone marrow transplantation (*10*). The significant interest in MSC based therapies is due to their convenient isolation, lack of significant immunogenicity (*10*−*12*), lack of ethical controversy, and potential to differentiate into tissue specific cell types. However, a significant barrier to the effective implementation of all cell therapies is the inability to deliver these cells under minimally invasive conditions with high efficiency of engraftment within tissues of interest. Systemic infusion is thus a desired mode of cell delivery; however, systemically administered culture expanded MSCs home at low efficiencies (*13*−*16*).

The white blood cells (i.e., leukocytes) and hematapoetic stem cells have evolved mechanisms for effective and specific tissue targeting (i.e., "homing" or "trafficking") that have been dissected at the cellular, molecular, and biophysical level. This includes a multistep adhesion and activation cascade that allows leukocytes to adhere to the luminal surface of the vascular endothelium and subsequently migrate into the underling tissues. It is well-established that transient rolling-type interactions (mediated by selectins) and transition to firm adhesion (mediated by integrins) are absolutely essential and limiting components of leukocyte and hematopoetic cell trafficking (*17*−*20*). The blood vessels in each tissue are known to exhibit particular surface properties that can direct the trafficking of circulating cells. In addition, upon an active stimulus such as inflammation, specific homing receptors or ligands are expressed on the surface of the endothelium within the inflamed tissue.

Current MSC transfusion experiments have shown that only subsets of MSCs have the capacity to home to specific tissues (*21*) and that MSCs may lose their capacity to home after cell culture (*22*). This is complicated by the heterogeneity in receptor expression on MSCs and the changes in receptor expression observed during culture (*23*−*25*). The limited homing efficiency of MSCs is likely due to a lack of relevant cell surface adhesion molecules.

The central hypothesis for this work is that surfaces of cells that exhibit poor homing capabilities such as culture expanded MSCs (*26*−*29*) can be engineered to possess leukocyte-like adhesion properties, without otherwise altering function, in order to promote more effective rolling, adhesion, and ultimately *in vivo* homing. The proof of principle for this hypothesis is provided by recent work by Sackstein et al., which showed enhanced bone marrow engraftment of systemically administered MSCs (*30*). Strategies for modification of cell surfaces to promote adhesion are typically elaborate and include genetic modifications, insertion of glycosylphosphatidylinositol-modified proteins into the membrane, and metabolic delivery of specific moieties to the cell surface (*31*). While it has been shown that cells modified using *N*-hydroxy-succinimide ester coupling to cell surface amine groups retain viability and the ability to be cultured (*32, 33*), this simple strategy has not been explored for promoting stem cell homing. Here, we demonstrate that it is possible to use this simple strategy to covalently couple the sialyl Lewis X (SLeX) moiety onto the surface of mesenchymal stem cells (MSCs) through biotin−streptavidin chemical modifications (Scheme 1) without compromising MSC viability, adhesion, proliferation, and multidifferentiation potential. We further show that this modification of SLeX homing receptors onto the surface of MSCs can promote a cell rolling response *in vitro* on substrates coated with P-selectin. A simple coupling scheme that is minimally invasive on the cell makes this approach a simple method to potentially target any cell type to specific tissues via the circulation.

The free amine groups present on the surface of cells are available to react with the *N*-hydroxy-succinimide group of biotinyl-*N*-hydroxy-succinimide to biotinylate the cell surface. To improve the biotinylation efficiency, sulfonated biotinyl-*N*-hydroxy-succinimide (sulfo-NHS-Biotin, BNHS) was used. BNHS is water-soluble, which limits its transport through the cell membrane and thus facilitates maximal interaction of the NHS group with the cell membrane. After biotinylating the MSC surface, cells were incubated with streptavidin to form

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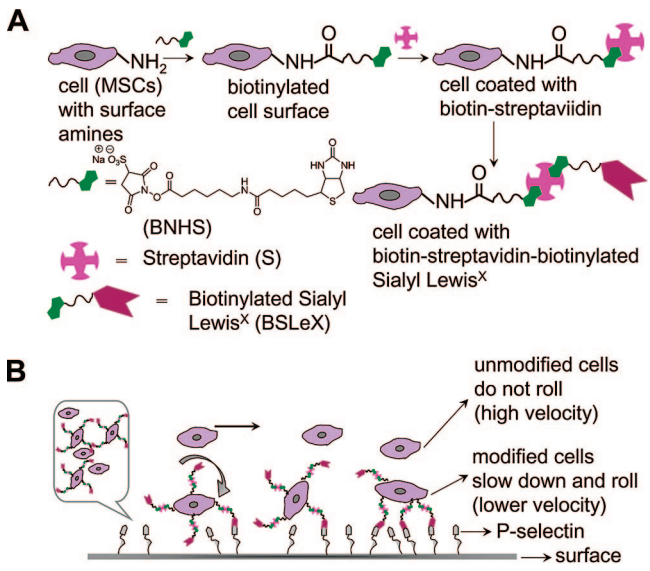
Scheme 1[a]



[a] (A) Modification of MSCs by SLeX using biotin−streptavidin. (B) Schematic presentation for rolling of SLeX modified MSCs on a P-selectin coated surface.

biotin−streptavidin complexes. The strong interaction between biotin and streptavidin stabilized the streptavidin on the cell surface. The streptavidin was then complexed with biotinylated-SLeX (sialyl-Lewis^X-PAA-Biotin, BSLeX; see Supporting Information) to introduce SLeX on the cell surface. It is believed that the absence of targeting agents such as SLeX on the surface of MSCs is responsible for their poor homing characteristics (*30*). Thus, we anticipated that synthetic immobilization of SLeX on the cell surface would induce a homing response through promoting cell rolling as shown in Scheme 1.

To assess the cell modification by BNHS and streptavidin, rhodamine-conjugated streptavidin (S) was added to biotinylated cells (BNHS+S), and incubated for 20 min. Subsequently, excess unbound S was removed by multiple washes with buffer, and the fluorescence intensity of the cells was measured. The controls used for this experiment were cells treated with only S (a control to assess the nonspecific adsorption of S), cells treated with both biotin (B) and S (B+S) to examine the need for covalent conjugation of biotin to the cell surface, and cells treated with only PBS to probe the impact of cell modifications on cell function and phenotype. The total fluorescence intensity was normalized to the group treated with PBS. All experiments were performed on ~80−90% confluent monolayers on tissue culture polystyrene, and fluorescence was only observed in regions with cells. Cells functionalized with BNHS (covalent attachment) had considerably higher fluorescence intensity compared to cells treated with B+S, indicating that cells that were covalently functionalized using the NHS moiety had greater amounts of streptavidin immobilized on their surfaces. After 7 days, the fluorescence intensity of BNHS+S treated cells was much higher compared to B+S and S controls (noncovalent and nonspecifically adsorbed, respectively), indicating that the conjugation of streptavidin to the biotinylated cells was efficient and stable (Figure 1A). By enumerating the number of DAPI stained nuclei with surrounding rhodamine fluorescence, after 7 days of the modification, the percentage of cells modified by BNHS+S was found to be 97 ± 1%, which was significantly higher than the controls B+S (10.5 ± 1.7%, $P = 0.004$) and S (2.3 ± 0.4%, $P = 0.008$). To assess the temporal accessibility of biotin on the cell surface, rhodamine−streptavidin was added

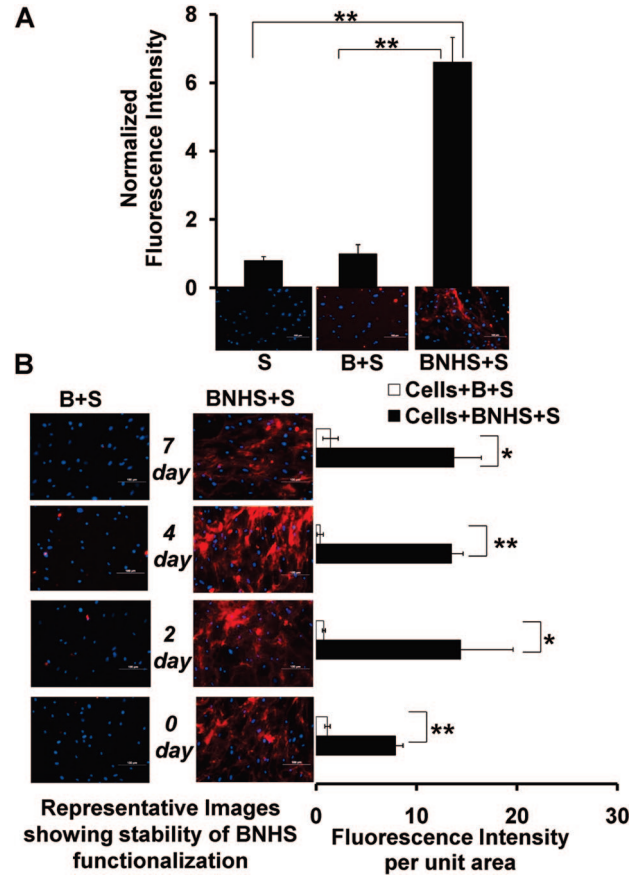


Figure 1. (A) Modification of the MSCs measured as a function of fluorescent signal 7 days after addition of S. (B) Accessibility (and stability) of covalently conjugated or adsorbed biotin on the MSC surface measured by addition of S at each time point.

at each time point to the biotinylated cells, and following multiple washes, the fluorescence intensity was measured (Figure 1B).

Specifically, MSCs were covalently functionalized with BNHS or functionalized via adsorption with native biotin (B) as a negative control. In both cases, experiments were conducted for 3 separate samples per group (triplicate) per time point. Each sample was processed and imaged. S was added on days 0, 2, 4, and 7, and after removing excess S by buffer washes, the fluorescence intensity was assessed (Figure 1B). An increase in the fluorescence intensity at day 2 compared with day 0 was observed, which may indicate the rearrangement and reorientation of the biotin moiety on the cell surface. The fluorescence intensity was stabilized and retained thereafter for up to 7 days and was significantly higher than the negative control at all time points ($P < 0.05$). This indicates the stability and accessibility of biotin on the cell surface via covalent immobilization chemistry. Presumably, streptavidin−biotin conjugated moieties, such as SLeX, would remain stably conjugated to the cell surface, accessible to interact with host biology. These results indicate the versatility and stability of the present approach.

To gain insight into the effect of covalent modification on cell characteristics such as viability, proliferation, adhesion, and ability to differentiate into multiple lineages, a series of experiments were performed. The cell viability was not considerably affected up to 48 h after BNHS+S modification compared to the control groups (Figure 2A). Specifically, 95% and 85% of PBS treated cells were viable after 0 and 48 h, respectively, whereas cells treated with B (noncovalent) were 81% and 76% viable and cells treated with BNHS (covalent) were 79% and 75% viable. This indicates that the BNHS+S

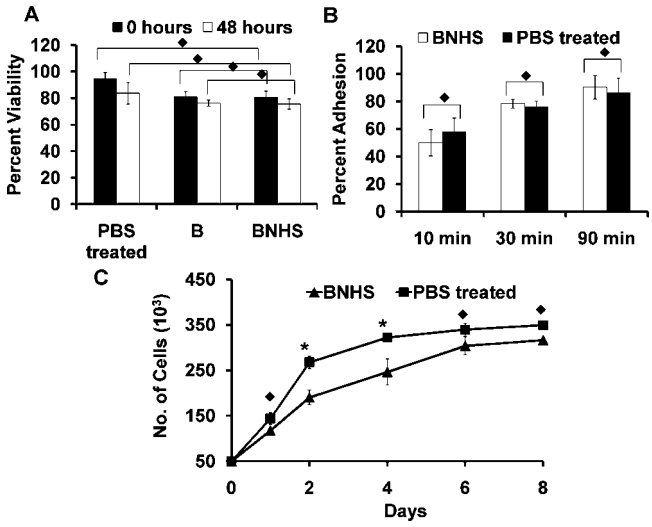


Figure 2. (A) Viability of the BNHS modified cells immediately after modification (0 h) and after 48 h. (B) Adherence of BNHS modified cells measured at 10, 30, and 90 min compared to the PBS treated cells. (C) Proliferation of the BNHS modified cells over an 8 day period compared to PBS treated cells. Error bars denote standard deviation from three separate experiments.

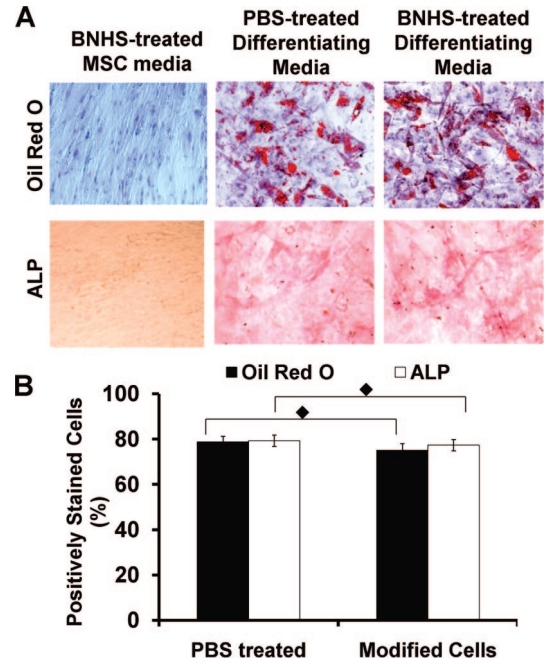


Figure 3. (A) Alkaline phosphatase (ALP) and oil red O (ORO) staining, 23 days after addition of osteogenic and adipogenic differentiation media, respectively. Negative controls (BNHS modified cells cultured in expansion media) showed no ORO or ALP staining. Positive controls (PBS treated cells in differentiating media) showed positive ORO and ALP staining. Experimental group (BNHS modified cells cultured in respective differentiating media) showed positive staining for both ORO and ALP. (B) Percentage of positively stained cells for ORO and ALP staining for PBS treated and BNHS modified cells. Error bars represent standard deviation from three separate treatments.

modification did not induce substantial cell toxicity. Cell adhesion characteristics were also not affected, as shown by the adhesion dynamics to tissue culture plastic of BNHS modified and PBS treated cells after 10, 30, and 90 min. This clearly indicates that the covalent modification of the cell surface did not compromise the ability of cells to interact with their extracellular environment (Figure 2B). On the contrary, BNHS modification inhibited the proliferation compared to the unmodified PBS treated cells on day 2 ($P = 0.01$, $n = 3$) and day 4 ($P = 0.02$, $n = 3$) (Figure 2C); however, both groups attained a confluent monolayer. This indicates that the covalent modification of the cell surface by biotin−streptavidin conjugation may have short-term negative impacts on proliferation but does not alter the normal substrate colonization characteristics of the MSCs.

Positive staining for oil red O (ORO) and alkaline phosphatase (ALP), indicators for adipogenic and osteogenic differentiation, respectively, was retained after BNHS modification (Figure 3; see Figure S1 in Supporting Information for higher magnification images). No differences in ORO or ALP staining were observed between BNHS modified cells and those treated with PBS after induction of osteogenic and adipogenic differentiation. Covalently modified MSCs cultured in nondifferentiating conditions (i.e., in MSC expansion medium) did not stain positively for osteogenic and adipogenic markers and the modification did not affect the number of positively stained cells compared to positive controls as shown in Figure 3B ($P > 0.05$, $n = 3$).

Taken together, the results suggest that BNHS modification of MSCs does not impair the potential for multilineage differentiation. However, it is important in future work to examine if other lineages are affected by the modification and if the trophic and immunomodulatory properties of MSCs are compromised. This work focused on examining the impact of cell surface modification with BNHS and S on cell function and phenotype. Although it is anticipated that the specific ligand attached through the streptavidin bridge would have a limited effect on these cell properties, each application would require extensive characterization for the candidate targeting ligand.

Since all experiments were performed with MSCs between passage 4 and 6, cell surface markers of passage 6 MSCs were examined by flow cytometry and immunofluorescence (data not shown). MSCs did not express hematopoetic markers CD34 or CD45 (0% of cells) but were shown to express high levels of MSC markers including CD90 and CD146 (100% of cells) as evidenced by flow cytometry. Immunofluorescence showed 0% expression of CXCR4 and 100% expression of MSC markers CD146, CD90, and CD271.

After examining the characteristic properties of MSCs with BNHS+S, BSLeX was introduced on cell surface as shown in Scheme 1, and its ability to impart a homing response was investigated via cell rolling within a standard parallel plate flow chamber system. Typically, culture expanded MSCs do not express ligands on their surface to enable cell rolling on selectin coated surfaces (*30*). MSCs modified with BNHS+S and biotinylated SLeX showed considerably lower velocities on immobilized P-selectin substrates compared to PBS treated cells ($P = 0.003$, $n = 3$) and cells treated with physically adsorbed BSLeX ($P = 0.04$, $n = 3$) (Figure 4A). Specifically, the interaction between SLeX and P-selectin reduced the velocity from ~65 μm/s to ~2 μm/s at a wall shear stress 0.366 dyne/cm^2. This clearly indicates that covalent modification of MSCs by biotin−streptavidin followed by SLeX−biotin induces a cell rolling response for culture expanded MSCs that is not typically observed.

The effect of flow rate and thus different shear stresses on the rolling characteristics is shown in Figure 4B,C. As shear stress is increased from 0.366 to 1.89 dyne/cm^2, the velocity of the modified cells increased whereas the number of cells interacting with P-selectin substrate remained relatively constant at ~75 cells/mm^2 (for 1 mM BNHS concentration) whereas the PBS treated cells displayed a near constant flux of ~20 cells/mm^2. This indicates that cells modified with SLeX interact with P-selectin substrates via recognized cell rolling based adhesion phenomena. The sharp increase in rolling velocity with increase in shear stress (Figure 4B), yet unchanged flux, may indicate

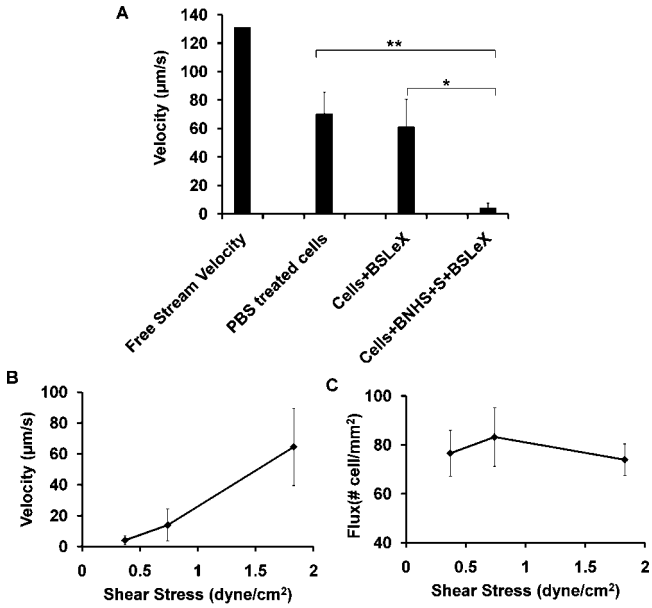


Figure 4. (A) Velocity of SLeX modified cells and controls on P-selectin treated surfaces at the shear stress of 0.366 dyne/cm^2. (B) Effect of shear stress on the rolling velocity. (C) Effect of shear stress on the number of interacting cells per unit area for the cells modified with SLeX.

that the SLeX is not uniformly distributed on the cell surface, or perhaps that the distribution of SLeX is unlike the clustering that typically appears on microvilli. Future work is thus required to characterize and optimize these parameters prior to *in vivo* homing studies. Together, this work demonstrates the potential to induce a leukocyte-like cell rolling response that may be useful to increase concentrations of MSCs and other cell types within specific tissues after systemic administration.

In conclusion, we have shown a novel, platform method for imparting a rolling response through functionalization of primary human MSCs with SLeX through combined covalent and biotin−streptavidin chemistries. Contrary to enzymatic modifications and retroviral transduction methods, the present approach is a simple, yet versatile method to engineer the cell membrane to induce a homing response. Importantly, covalently modified cells showed similar viability, proliferation, adhesion, and differentiation potential (as judged by ORO and ALP staining for adipogenic and osteogenic differentiation, respectively) compared to PBS treated cells. Apart from the specific interactions between SLeX and P-selectin to induce a rolling response, there are several other important cellular and molecular features that are crucial to induce and sustain cell trafficking behavior including cell deformability and cell−substrate contact area that is typically dictated by microvillus extensions (*33*), firm adhesion, and transmigration. Future work will involve characterizing the impact of the covalent immobilization methodology on these parameters and investigating coimmobilization of other adhesion molecules to further enhance the potential for an effective *in vivo* homing response. It would also be important to characterize the impact of engineered MSCs on the latter stages of cell homing that involve transendothelial migration and chemotaxis in response to soluble cues. We anticipate that the present approach to covalently modify the cell surface and immobilize required ligands is not limited to MSCs or the SLeX ligand. Hence, this technology should have broad implications on cellular therapies that utilize systemic administration and require targeting of cells to specific tissues.

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Supporting Information Available: Detailed materials and methods, fluorescent measurements and images of ORO and ALP staining. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Gastineau, D. A. (2004) Will regulation be the death of cell therapy in the United States? *Bone Marrow Transplant 33*, 777–80.

(2) Picinich, S. C., Mishra, P. J., Mishra, P. J., Glod, J., and Banerjee, D. (2007) The therapeutic potential of mesenchymal stem cells. Cell- & tissue-based therapy. *Expert Opin. Biol. Ther. 7*, 965–73.

(3) Burt, R. K., Loh, Y., Pearce, W., Beohar, N., Barr, W. G., Craig, R., Wen, Y., Rapp, J. A., and Kessler, J. (2008) Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. *JAMA 299*, 925–36.

(4) Hashemi, S. M., Ghods, S., Kolodgie, F. D., Parcham-Azad, K., Keane, M., Hamamdzic, D., Young, R., Rippy, M. K., Virmani, R., Litt, H., and Wilensky, R. L. (2008) A placebo controlled, dose-ranging, safety study of allogenic mesenchymal stem cells injected by endomyocardial delivery after an acute myocardial infarction. *Eur. Heart J. 29*, 251–9.

(5) Abdel-Latif, A., Bolli, R., Tleyjeh, I. M., Montori, V. M., Perin, E. C., Hornung, C. A., Zuba-Surma, E. K., Al-Mallah, M., and Dawn, B. (2007) Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch. Intern. Med. 167*, 989–97.

(6) Sykova, E., Jendelova, P., Urdzikova, L., Lesny, P., and Hejcl, A. (2006) Bone marrow stem cells and polymer hydrogels--two strategies for spinal cord injury repair. *Cell Mol. Neurobiol. 26*, 1113–29.

(7) Filho Cerruti, H., Kerkis, I., Kerkis, A., Tatsui, N. H., da Costa Neves, A., Bueno, D. F., and da Silva, M. C. (2007) Allogenous bone grafts improved by bone marrow stem cells and platelet growth factors: clinical case reports. *Artif. Organs 31*, 268–73.

(8) Gupta, M. C., Theerajunyaporn, T., Maitra, S., Schmidt, M. B., Holy, C. E., Kadiyala, S., and Bruder, S. P. (2007) Efficacy of mesenchymal stem cell enriched grafts in an ovine posterolateral lumbar spine model. *Spine 32*, 720–6, discussion 727.

(9) Garcia-Olmo, D., Garcia-Arranz, M., Herreros, D., Pascual, I., Peiro, C., and Rodriguez-Montes, J. A. (2005) A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis. Colon Rectum 48*, 1416–23.

(10) Maitra, B., Szekely, E., Gjini, K., Laughlin, M. J., Dennis, J., Haynesworth, S. E., and Koc, O. N. (2004) Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. *Bone Marrow Transplant 33*, 597–604.

(11) Aggarwal, S., and Pittenger, M. F. (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood 105*, 22–1815.

(12) Le Blanc, K., Tammik, L., Sundberg, B., Haynesworth, S. E., and Ringden, O. (2003) Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand. J. Immunol. 57*, 11–20.

(13) Zhang, M., Mal, N., Kiedrowski, M., Chacko, M., Askari, A. T., Popovic, Z. B., Koc, O. N., and Penn, M. S. (2007) SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. *Faseb J. 21*, 3197–207.

(14) Barbash, I. M., Chouraqui, P., Baron, J., Feinberg, M. S., Etzion, S., Tessone, A., Miller, L., Guetta, E., Zipori, D., Kedes, L. H., Kloner, R. A., and Leor, J. (2003) Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation 108*, 863–8.

(15) Chen, J., Li, Y., Wang, L., Zhang, Z., Lu, D., Lu, M., and Chopp, M. (2001) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke 32*, 1005–11.

(16) Kawada, H., Fujita, J., Kinjo, K., Matsuzaki, Y., Tsuma, M., Miyatake, H., Muguruma, Y., Tsuboi, K., Itabashi, Y., Ikeda, Y., Ogawa, S., Okano, H., Hotta, T., Ando, K., and Fukuda, K. (2004) Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. *Blood 104*, 3581–7.

(17) Lawrence, M. B., and Springer, T. A. (1991) Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell 65*, 859–73.

(18) Salas, A., Shimaoka, M., Chen, S., Carman, C. V., and Springer, T. (2002) Transition from rolling to firm adhesion is regulated by the conformation of the I domain of the integrin lymphocyte function-associated antigen-1. *J. Biol. Chem. 277*, 50255–62.

(19) Ley, K., Laudanna, C., Cybulsky, M. I., and Nourshargh, S. (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol. 7*, 678–89.

(20) Greenberg, A. W., Kerr, W. G., and Hammer, D. A. (2000) Relationship between selectin-mediated rolling of hematopoietic stem and progenitor cells and progression in hematopoietic development. *Blood 95*, 478–86.

(21) Wynn, R. F., Hart, C. A., Corradi-Perini, C., O'Neill, L., Evans, C. A., Wraith, J. E., Fairbairn, L. J., and Bellantuono, I. (2004) A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood 104*, 2643–2645.

(22) Rombouts, W. J., and Ploemacher, R. E. (2003) Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. *Leukemia 17*, 160–70.

(23) Meirelles, L. d. S., Chagastelles, P. C., and Nardi, N. B. (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J. Cell. Sci. 119*, 2204–2213.

(24) Jones, E. A., Kinsey, S. E., English, A., Jones, R. A., Straszynski, L., Meredith, D. M., Markham, A. F., Jack, A., Emery, P., and McGonagle, D. (2002) Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. *Arthritis Rheum. 46*, 3349–60.

(25) Simmons, P. J., and Torok-Storb, B. (1991) Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood 78*, 55–62.

(26) Barbash, I. M., Chouraqui, P., Baron, J., Feinberg, M. S., Etzion, S., Tessone, A., Miller, L., Guetta, E., Zipori, D., Kedes, L. H., Kloner, R. A., and Leor, J. (2003) Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation 108*, 863–868.

(27) Chen, J., Li, Y., Wang, L., Zhang, Z., Lu, D., Lu, M., and Chopp, M. (2001) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke 32*, 1005–1011.

(28) Kawada, H., Fujita, J., Kinjo, K., Matsuzaki, Y., Tsuma, M., Miyatake, H., Muguruma, Y., Tsuboi, K., Itabashi, Y., Ikeda, Y., Ogawa, S., Okano, H., Hotta, T., Ando, K., and Fukuda, K. (2004) Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. *Blood 104*, 3581–3587.

(29) Jiang, W., Ma, A., Wang, T., Han, K., Liu, Y., Zhang, Y., Zhao, X., Dong, A., Du, Y., Huang, X., Wang, J., Lei, X., and Zheng, X. (2006) Intravenous transplantation of mesenchymal stem cells improves cardiac performance after acute myocardial ischemia in female rats. *Transplant Int. 19*, 570–580.

(30) Sackstein, R., Merzaban, J. S., Cain, D. W., Dagia, N. M., Spencer, J. A., Lin, C. P., and Wohlgemuth, R. (2008) Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat. Med. 14*, 181–187.

(31) Mahal, L. K., and Bertozzi, C. R. (1997) Engineered cell surfaces: Fertile ground for molecular landscaping. *Chem. Biol. 4*, 415–422.

(32) Hausmann, S., Claus, R., and Walzel, H. (1995) Short-term culture of surface-biotinylated cells: Application in non-radioactive analysis of surface protein shedding. *Immunol. Lett. 48*, 175–180.

(33) Yago, T., Leppanen, A., Qiu, H., Marcus, W. D., Nollert, M. U., Zhu, C., Cummings, R. D., and McEver, R. P. (2002) Distinct molecular and cellular contributions to stabilizing selectin-mediated rolling under flow. *J. Cell. Biol. 158*, 99–787.

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