Biomaterials 31 (2010) 5266–5274

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Engineered mesenchymal stem cells with self-assembled vesicles for systemic cell targeting

Debanjan Sarkar [a], Praveen K. Vemula [a], Weian Zhao [a], Ashish Gupta [a,1], Rohit Karnik [b], Jeffrey M. Karp [a,*]

[a] Harvard-MIT Division of Health Sciences and Technology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Harvard Stem Cell Institute, 65 Landsdowne Street, Cambridge, MA 02139, USA
[b] Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

ARTICLE INFO

Article history:
Received 23 February 2010
Accepted 3 March 2010
Available online 8 April 2010

Keywords:
Self-assembly
Mesenchymal stem cell
Inflammation

ABSTRACT

Cell therapy has the potential to impact the quality of life of suffering patients. Systemic infusion is a convenient method of cell delivery; however, the efficiency of engraftment presents a major challenge. It has been shown that modification of the cell surface with adhesion ligands is a viable approach to improve cell homing, yet current methods including genetic modification suffer potential safety concerns, are practically complex and are unable to accommodate a wide variety of homing ligands or are not amendable to multiple cell types. We report herein a facile and generic approach to transiently engineer the cell surface using lipid vesicles to present biomolecular ligands that promote cell rolling, one of the first steps in the homing process. Specifically, we demonstrated that lipid vesicles rapidly fuse with the cell membrane to introduce biotin moieties on the cell surface that can subsequently conjugate streptavidin and potentially any biotinylated homing ligand. Given that cell rolling is a pre-requisite to firm adhesion for systemic cell homing, we examined the potential of immobilizing sialyl Lewis X (SLeX) on mesenchymal stem cells (MSCs) to induce cell rolling on a P-selectin surface, under dynamic flow conditions. MSCs modified with SLeX exhibit significantly improved rolling interactions with a velocity of 8 μm/s as compared to 61 μm/s for unmodified MSCs at a shear stress of 0.5 dyn/cm^2. The cell surface modification does not impact the phenotype of the MSCs including their viability and multi-lineage differentiation potential. These results show that the transitory modification of cell surfaces with lipid vesicles can be used to efficiently immobilize adhesion ligands and potentially target systemically administered cells to the site of inflammation.

1. Introduction

Cell therapy holds enormous promise for the treatment of many diseases and disorders. In particular, mesenchymal stem cells (MSCs), also referred to as multipotent mesenchymal stromal cells, are used for minimally invasive cell therapy to promote regeneration of damaged tissue, to treat inflammation and to promote angiogenesis [1]. The potential clinical utility of MSCs for regenerative therapeutics is due to their easy isolation, lack of immunogenic response (permitting allogeneic transplantation without immunosuppressive drugs), ability to down-regulate inflammation, and their potential to promote vascularization or differentiate into multiple lineages [2–5]. Thus, MSCs are being considered to regenerate damaged tissue and treat inflammation resulting from graft-versus-host disease, cardiovascular disease and myocardial infarction, brain and spinal cord injury, cartilage and bone injury and Crohn's disease [6].

However, a major challenge in MSC therapy and cell therapy in general, is to efficiently and minimally invasively deliver therapeutic cells to a target location [7]. The delivery of MSCs via systemic infusion is a desirable route being explored in clinic trials because local transplantation is highly invasive and locally administered cells die due to lack of nutrients and oxygen [7,8]. Unfortunately, only a small percentage (typically less than 1%) of culture expanded MSCs can home to the tissue of interest, which is due to the absence of some of the key homing receptors that are typically utilized by hematopoietic stem cells (HSCs) and leukocytes [7,9]. MSCs either do not possess, or lose the cell surface expression of homing receptors during culture expansion [10,11]. For efficient homing of systemically delivered stem cells, one may target the endothelium within the tissue of interest by mimicking the adhesion cascade of HSCs and leukocytes. Cell rolling is a critical

* Corresponding author. Tel.: +1 617 817 9174; fax: +1 617 768 8338.
E-mail address: jkarp@rics.bwh.harvard.edu (J.M. Karp).
URL: http://www.karplab.net
[1] Present address: Genomics, BioInnovationsZentrum, Technische Universität Dresden, Dresden, Germany.

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.03.006

step of this adhesion cascade and is mediated by selectins expressed on the endothelium of the target organ or tissue [12,13]. Rolling interactions mediate rapid deceleration of cells from the blood-stream to initiate subsequent steps in the adhesion cascade including firm adhesion and transendothelial migration into the tissue [14,15]. A potential solution to enhance targeted engraftment of MSCs is therefore to promote the expression of homing ligands on their membrane. Modification of MSCs with retrovirus vectors encoding homing receptors such as CXCR4, or the α4 subunit of the VLA-4-integrin, has been recently used to enhance homing of MSCs [16–18]. In addition to genetic modification, Sackstein et al. have recently shown engineering of CD44 by enzymatic modification on MSC membrane to hematopoietic cell E-selectin/L-selectin ligand (HCELL) enables them to home to bone marrow more efficiently than unmodified MSCs [19]. Another approach involves the conjugation of antibodies to the cell surface via bispecific antibodies or palmitated protein G or protein A, which permits cell surface functionalization by any antibody with an accessible Fc region [20,21]. However, such firm adhesion ligands are typically not suitable to promote homing given that an initial rolling interaction is a pre-requisite for firm adhesion [22,23]. Thus, a simple and versatile method is required to introduce adhesion ligands on the cell surface without the risks associated with genetic modification or complexity of enzymatic techniques.

Recently, we developed a versatile platform technology to covalently attach cell adhesion molecules to the cell surface to improve homing efficiency to specific tissues [24]. Specifically, we immobilized sialyl Lewis X (SLeX), the active site of P-selectin glycoprotein ligand-1 (PSGL-1) found on leukocytes, on the surface of MSCs with biotin–streptavidin bridge and showed that MSCs exhibit a rolling response on a P-selectin coated substrate under shear stress conditions, indicating their potential utility in targeting P-selectin expressing endothelium in the bone marrow or at sites of inflammation. As we showed, this approach offers a method to modify the cell surface for longer duration in a non-specific manner. We wanted to design a system where the adhesion ligands are introduced only transiently for short duration allowing for initial adhesion and cytokine activation, not impeding subsequent extravasation. Therefore, to develop an approach that offers a transient cell surface modification and avoids non-specific modification of cell surface molecules, we envisioned the immobilization of ligands through coalescence of ligand-carrying lipid vesicles with the cell membrane. In this work, we have demonstrated that unilamellar vesicles composed of biotinylated lipid can be utilized to anchor biotin on the MSCs cell membrane through intercalation of the lipid vesicles with lipid bilayer of the cell membrane (Fig. 1). Subsequently, biotinylated sialyl Lewis X (SLeX) was immobilized on the cell surface through a biotin–streptavidin bridge, by treating biotinylated MSCs with streptavidin followed by biotinylated SLeX (Fig. 2A). SLeX binds to selectins on endothelium and is utilized by leukocytes and HSCs to promote tethering and rolling interactions on endothelium under the hydrodynamic conditions of blood flow (Fig. 2B). The SLeX–selectin interactions subsequently lead to firm adhesion and transmigration through vessel wall into tissue. This method presents an alternative cell membrane engineering approach to introduce a ligand of interest on the cell membrane for short duration, in contrast to enzymatic and covalent modification methods. Furthermore, this approach offers a platform that can be used to investigate engineered stem cell homing and interrogate the biology of cell homing.

2. Materials and methods

2.1. Materials

Biotinylated lipid 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(biotinyl) (sodium salt) was purchased from Avanti Polar Lipids (Alabaster, AL). P-selectin was purchased from R&D systems (Minneapolis, MN) and biotinylated sialyl Lewis (X)-poly(acrylamide) (sialyl Lewis X-PAA-Biotin) and biotinylated glucose-poly(acrylamide) was purchased from Glycotech (Gaithersburg, MD). Primary human MSCs were obtained from the Center for Gene Therapy at Tulane University which has a grant from NCRR of the NIH, Grant #P40RR017447. α-MEM, L-Glutamine and Penn–Strep were purchased from Invitrogen. Fetal Bovine Serum was purchased from Atlanta Biologicals (Lawrenceville, GA) and Rhodamine–streptavidin was purchased from Fisher Scientific (Waltham, MA). All other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO) and used without further modification unless specified. MSCs were maintained in expansion medium that included 15% Fetal Bovine Serum (selected for its ability to expand MSCs), 1% (v/v) L-Glutamine, 1% (v/v) Penn–Strep, and α-MEM. All experiments were performed using MSCs at passage number 4–6 where cells expressed high level MSC markers CD90 and CD29 (>99% cells), and did not express hematopoietic markers CD34 or CD45 (0% of cells) as observed from flow cytometry analysis (results not shown).

2.2. Preparation of vesicles

Chloroform solution containing the biotinylated lipid 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(biotinyl) Bio-PE (50 μL from 10 mg/mL stock solution) was transferred to a glass vial and chloroform was slowly evaporated under nitrogen, and residual solvent was removed by storing the dried lipid under vacuum for at least 4 h prior to use. The resulting dry film was hydrated with 5 mL of PBS for 24 h at 4 °C, and subjected to three cycles of freeze/thaw/vortex and sonication to produce unilamellar vesicles.

2.3. Transmission electron microscopy (TEM) and dynamic light scattering (DLS)

Two drops of 1 mM vesicle solution was placed on Cu-grid, to which 1 drop of negative staining agent phosphotungstic acid (2 wt%) was added. Excess aqueous solution was removed with a kimwipe. After drying at ambient conditions, grids were examined using a Jeol 200CX TEM (operated at 120 kV). 3 mL Of vesicle solution (1 mM) was taken in a quartz cuvette and size and zeta potential analysis was performed using dynamic light scattering (ZEN 3690, Malvern Instrument, Inc.).

2.4. Modification of MSCs with biotinylated vesicles

MSCs were seeded in tissue culture wells of 96-well plates and were cultured in a cell expansion media until reaching 90% confluence. 0.2 mL Of 1 mM vesicle solution in cell expansion media was added to the adherent MSCs on tissue culture plates and incubated for 10 min at room temperature and washed subsequently with 200 μL of media thrice. Subsequently, rhodamine–streptavidin solution (50 μg/mL in PBS, pH 7.4 without Ca/Mg) was added and incubated for 5 min. Cells were then washed twice with PBS to remove unbound rhodamine–streptavidin. The cells were imaged using fluorescence microscopy to assess the cell surface modification by analyzing the red fluorescence from rhodamine. For control experiments, MSCs were treated with only biotin (without lipid molecule/vesicle-form) followed by incubation with rhodamine–streptavidin solution. Rhodamine–streptavidin conjugated to vesicle modified MSCs were characterized with Zeiss LSM510 laser scanning confocal microscope at 100× of the lens. Modified MSCs were fixed with 3.7% formaldehyde at room temperature and stained with 50 μg/mL of phalloidin-FITC solution for 30 min to visualize F-actin and the nuclei were stained with 10 μM DRAQ5® (Cell Signaling Technology, MA) in PBS for 5 min.

The stability of the modification was examined after 8 h through measurement of the fluorescent signal from rhodamine–streptavidin conjugated to the vesicle modified MSCs. In addition, the stability of rhodamine–streptavidin conjugated to vesicle modified MSCs were characterized using laser scanning confocal microscopy at 100×.

2.5. Accessibility of biotinylated lipid molecules

To characterize the accessibility of the biotin on the vesicle modified cell surface, 0.2 mL of rhodamine–streptavidin (50 μg/mL in PBS, pH 7.4 without Ca/Mg) was added for 5 min at room temperature on either biotin or vesicle treated cells after designated time-points. After addition of rhodamine–streptavidin, the cells were fixed with 3.7% formaldehyde solution and stained with 100 μL of DAPI solution (1 μg/mL in PBS) after treatment with 100 μL of 0.1% TRITON × solution in PBS. The fluorescence intensity of microscopic images was analyzed using Nikon quantitative software (NIS-Elements version 3).

2.6. Rolling interactions of vesicle modified cells

2.6.1. Preparation of P-selectin surfaces

P-selectin (1 mL of a 5 μg/mL solution) was placed on 35 mm tissue culture petridish and incubated on a plate shaker for 18 h at room temperature. All P-selectin surfaces were prepared freshly before the flow chamber assay.

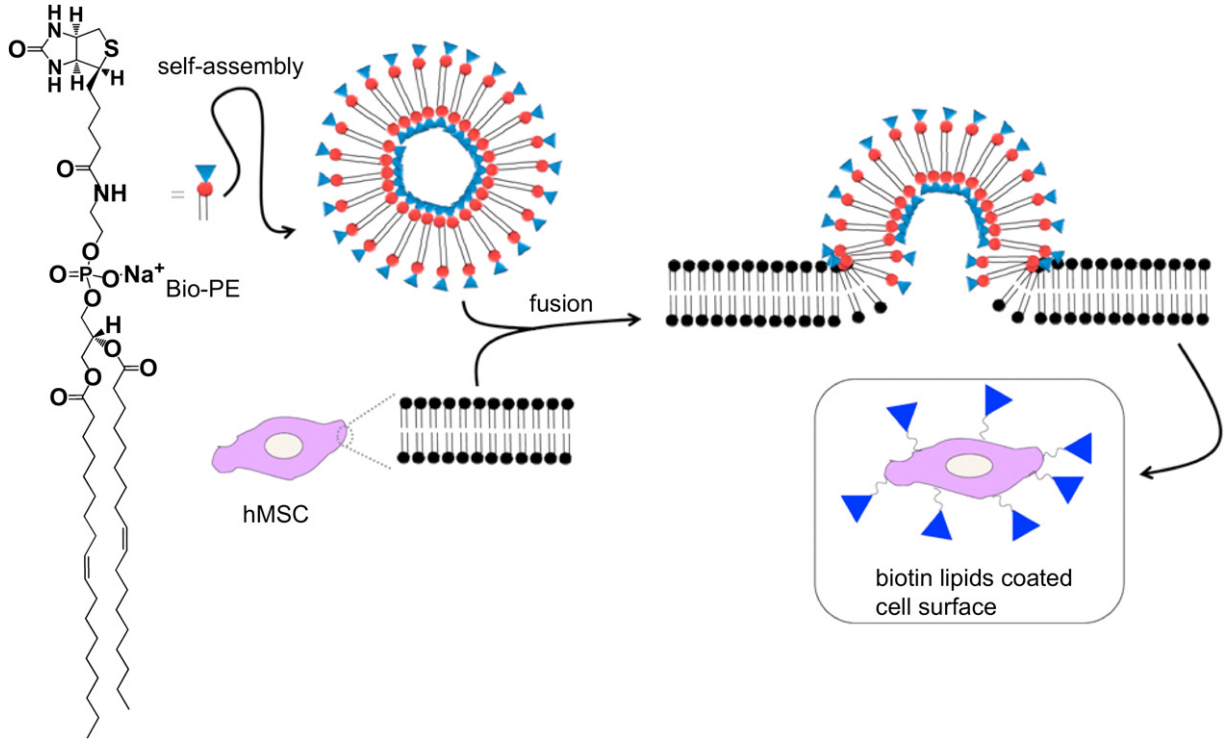

Fig. 1. Schematic showing formation of vesicles from biotinylated lipid and modification of MSCs with biotinylated lipid vesicles.

2.6.2. Flow chamber assay

For flow chamber experimentation, the cells were biotinlyated with vesicle solution as described above in a T25 flask. 1 mL Of streptavidin solution (50 μg/mL in PBS, pH 7.4 without Ca/Mg) was added for 5 min at room temperature to vesicle treated cells. Cells were washed twice with 1 mL of phosphate buffer saline (PBS, pH 7.4 without Ca/Mg) at room temperature followed by addition of 1 mL of 5 μg/mL biotinylated SLeX (Biotin-PAA-SLeX in PBS, pH 7.4 without Ca/Mg) at room temperature for 5 min. The cells were detached from the flask by using 1 mL of cell dissociation solution and then re-suspended in MSC expansion media ($\sim 10^5$ cells/mL) for analysis of cell velocities within a parallel plate flow chamber. To examine the effect of biotinylated lipids on the rolling response, MSCs were modified with biotinylated lipid vesicles only in the first step and then detached from the flask by using cell dissociation buffer. To examine the specificity of SLeX as a rolling ligand, MSCs were modified with glucose, a monosaccharide as a non-rolling ligand. MSCs modified with biotinylated vesicles and streptavidin were incubated with 1 mL of 5 μg/mL biotinylated Glucose (Biotin-PAA-Glucose in PBS, pH 7.4 without Ca/Mg) at

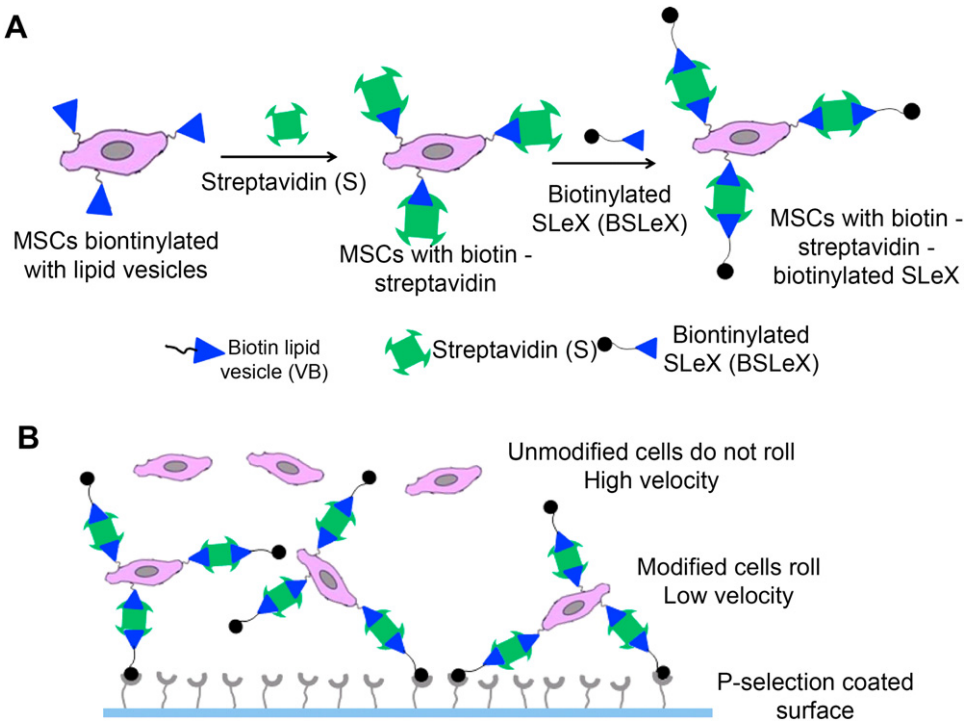

Fig. 2. (A) Modification of vesicle modified MSCs by SLeX using streptavidin and biontinylated SLeX. (B) Rolling of SLeX modified MSCs on a P-selectin coated surface.

room temperature. The cells were detached from the flask by using 1 mL of cell dissociation solution and then re-suspended in MSC expansion media. Additionally, SLeX modified MSCs were pre-incubated with P-selectin to block the SLeX from interacting with P-selectin. SLeX modified MSCs in the flask were incubated with 1 mL of 50 μg/mL P-selectin (in PBS, pH 7.4 without Ca/Mg) at room temperature for 10 min. The cells were detached from the flask by using 1 mL of cell dissociation solution and then re-suspended in MSC expansion media.

The rolling characteristics of the cells were assessed using a circular parallel plate flow chamber (Glycotech, Gaithersburgh, MD) with 127 μM gasket thickness. For the majority of experiments, a flow rate corresponding to a wall shear stress of 0.5 dyne/cm^2 was used. To monitor cell rolling, phase contrast microscopy (TE2000-U Inverted Nikon Microscope with a DS-Qi1 Monochrome Cooled Digital Camera) was utilized and images were recorded in a 10× field at 10 s intervals. The velocity of the cells was calculated by measuring the distance cells travelled within a 10 s interval. A cell was classified as rolling if it rolled for 10 s while remaining in the field of view and if it translated at an average velocity less than 50% of the calculated free stream velocity of a non-interacting cell. PBS treated cells were used as control for this experiment. The number of interacting cells was calculated manually based on number of total cells remaining (either rolling or adherent) within the field view for 10 s. To assess the effect of shear rate, the rolling velocity and the number of interacting cells were measured at flow rates corresponding to 0.5, 0.75 and 1.0 dyne/cm^2, respectively.

2.7. Viability, adhesion and proliferation characteristics

Cell viability, adhesion and proliferation were examined after modifying the cells with vesicles followed by streptavidin and biotinylated SLeX. Briefly, cells were plated into 6-well plates, left to adhere overnight followed by treatment with 1 mL of 1 mM vesicle solution for 10 min. After incubation the wells were washed with media thrice and 1 mL streptavidin solution (50 μg/mL in PBS, pH 7.4 without Ca/Mg) was added to each well. The wells were incubated with streptavidin solution for 5 min, washed twice with PBS, followed by addition of 1 mL of biotinylated SLeX (5 μg/mL in PBS, pH 7.4 without Ca/Mg) to each well. After 5 min, the wells were washed twice with PBS, the cells were detached with 400 μL of cell dissociation solution and the viability of the cells was examined using trypan blue exclusion. Cell viability was also examined after 48 h incubation at 37 °C and 5% CO_2. The media was then aspirated (similar quantities of floating cells were observed between the groups – typically 1–5 per well) and the cells were detached with 400 μL of cell dissociation solution. 600 μL Of media was added and the total 1 mL of the cell dispersion was collected in an eppendorf tubes. From this 10 μL of cell dispersion was diluted to 1:1 with 4% trypan blue solution and cells were counted in a hemocytometer to determine the number of viable (non-blue) and non-viable (blue) cells. PBS treated cells served as a control. Cell adhesion was quantified by measuring the number of adherent cells on tissue culture surfaces in 96-well plates at 10, 30 and 90 min after modifying the cells as described above. 5000 Cells were added to each well of a 96-well plate in 200 μL of MSC cell expansion media followed by incubation at 37 °C. The non-adhered cells were removed by rinsing with PBS. The adhered cells were manually counted under microscope at 10× in three random fields for three replicates. The adherence was expressed as the percentage of adhered cells. PBS treated cells served as a control. Proliferation of the cells was quantified by calculating the doubling time for the cells within a T25 flask by counting the cells with light microscopy at 10×. Doubling time was calculated from the linear regression of the cell growth curve.

2.8. Differentiation of cells

Multi-lineage differentiation potential of the vesicle modified and PBS treated cells was examined by treating cells with osteogenic and adipogenic induction media followed by respective colorimetric histological staining.

2.8.1. Osteogenic differentiation

MSCs seeded in the wells of 24-well plates were cultured in MSC expansion media until reaching 90% confluence. For cell modification, cells were biotinylated with 1 mM vesicle solution followed by conjugation with streptavidin (50 μg/mL) in PBS and biotinylated SLeX (5 μg/mL) in PBS at room temperature. Osteogenic differentiation was induced by culturing the cells for 23 days in osteogenic induction media (from Lonza – MSCs Osteogenic Single Quote kit) containing dexamethasone, β-glycerophosphate, L-ascorbic acid-2-phosphate, and α-MEM. For a positive control, PBS treated (unmodified) cells were maintained in osteogenic induction medium and for a negative control, vesicle modified cells were maintained in MSC expansion media. The media in both groups was changed every 3 days. Osteogenesis was evaluated by cell membrane associated alkaline phosphatase activity. Alkaline phosphatase activity was examined after 23 days by carefully aspirating the medium and washing the cells with distilled water. The cells were fixed with 3.7% formaldehyde solution for 15 min at room temperature followed by rinsing with distilled water. 0.06% Red Violet LB salt solution in Tris HCl was added with distilled water containing DMF and Naphthol AS MX-PO_4. The plates were incubated for 45 min and then the wells were rinsed three times with distilled water and visualized with light microscopy.

2.8.2. Adipogenic differentiation

MSCs seeded in 4 wells of a 24-well plate were cultured in cell expansion media until reaching 100% confluence. Adipogenic differentiation was induced by culturing the cells for 23 days in adipogenic induction media (from Lonza – MSCs Adipogenic Single Quote kit containing h-Insulin (recombinant), L-Glutamine, Dexamethasone, Indomethacin, IBMX (3-isobuty-l-methyl-xanthine), Pen/Strep) and adipogenic maintenance media (from Lonza – MSCs Adipogenic Single Quote kit containing h-Insulin (recombinant), L-Glutamine, Pen/Strep). As a positive control, PBS treated (unmodified) cells were maintained in adipogenic induction medium, and as a negative control the vesicle modified cells were maintained in normal MSC expansion media. The media in both groups was changed every 3 days in a periodic exposure of induction and maintenance media as suggested by the supplier (Lonza). Adipogenesis was evaluated by Oil Red O staining. Following aspiration of the media, cells were washed once with PBS, and fixed in PBS with 3.7% formaldehyde for 30 min at room temperature. Following fixation cells were rinsed with distilled water for a few minutes, and incubated with isopropanol containing an Oil Red O working solution (prepared by diluting 30 mL of 0.5% isopropanol/Oil Red O solution with 20 mL of distilled water). After 45 min culture well were rinsed twice with distilled water and 1 mL of hematoxylin was added to each well for 1 min followed by aspiration and washing with distilled water. The wells were viewed using an inverted phase contrast microscope. Lipid droplets appeared red and nuclei appeared blue.

3. Results and discussion

3.1. Characterization of vesicles and anchoring of lipids into cell membrane

Unilamellar vesicles were prepared using amphiphilic biotinylated lipid (Bio-PE) through a freeze-thaw method [25]. Due to the amphiphilicity of lipids, they self-assemble into lamellar structures at room temperature (Fig. 1). The morphology of these vesicles was generally spherical and unilamellar, as characterized by transmission electron microscope (TEM) images (Fig. 3A). TEM images show that width of vesicle membrane is approximately 5.1 nM, which is higher than the molecular length of biotinylated lipid (3.1 nM from the optimized geometry calculations) and much lower than the double of extended molecular length of lipid. This suggests the formation of unilamellar vesicles through highly interdigitated bilayer membrane. The hydrodynamic size of vesicles, measured using dynamic light scattering (DLS), was determined to be ~98 nM with a heterogeneous distribution (Fig. 3B)

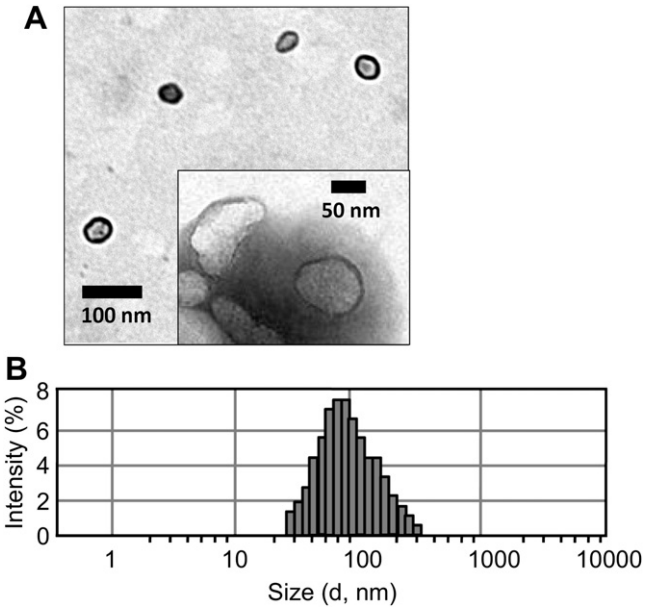

Fig. 3. (A) Transmission electron microscopic images of biotinylated lipid vesicles (inset shows higher magnification image of vesicles with unilamellar structure). (B) Size distribution analysis of vesicles with dynamic light scattering showing non-uniform distribution of sizes with an average vesicle diameter of 98 nM.

which is consistent with the size observed through TEM. The Bio-PE vesicles were negatively charged, −25.9 mV, as determined using zeta potential analysis.

It is known that self-assembled vesicles, such as fatty acid containing amphiphiles, undergo spontaneous fusion with the cell membrane, which results in the insertion of lipid molecules into the cell membrane [26]. In the present work, the MSC surface was biotinylated through modification with unilamellar vesicles of biotinylated lipid at room temperature. The presence of biotin on cell membrane after modification was assessed by examining the fluorescent signal of rhodamine–streptavidin. Fig. 4A shows significantly higher fluorescence signal for vesicle modified cells compared to controls where the cells were either treated with biotin followed by rhodamine–streptavidin or treated with only rhodamine–streptavidin. This indicates that the biotin assembled on the surface of the MSCs is accessible for conjugation of streptavidin. Confocal images (Fig. 4B) show the presence of fluorescent streptavidin which verified the presence of fused biotinylated lipid on the MSC surface immediately after modification (0 h). Co-localization of green-fluorescence signal from phalloidin-FITC stained actin, blue-fluorescence from DRAQ5 stained nucleus and red fluorescence from rhodamine–streptavidin conjugated to biotinylated lipid was used to confirm that streptavidin was present on the surface of the cells. Interestingly, we have observed that immediately after modification (Fig. 4B, 0 h) the streptavidin on the cell surface is clustered which could be due to the fusion of vesicles with cell membrane resulting in locally clustered ligand domains on cell membrane. These domains can be formed due to organization of the fused lipid vesicles as rafts and can be stabilized by the complexation between biotin and streptavidin [27,28].

The stability of streptavidin on the surface of the vesicle modified MSCs was investigated by examining the fluorescence signal of rhodamine–streptavidin with immunofluorescence (Fig. 4B). 8 h after the modification, the fluorescence intensity of the streptavidin was decreased by ~50%. The reduction of fluorescent signal of rhodamine–streptavidin indicates the loss of conjugated streptavidin from cell. This loss of streptavidin signal can be partly due to the quenching of fluorescence of the internalized streptavidin by the local environmental factors (i.e. pH, metal ions, etc) inside the cells [29]. Additionally, the distribution of residual streptavidin after 8 h was examined with confocal microscopy. The confocal images (Fig. 4B) show that a fraction of streptavidin was present on the surface of MSCs as clusters after 8 h and a fraction of the conjugated streptavidin was internalized. The clustering of ligands on the cell surface may present advantages for engaging specific ligands on endothelium, compared to conjugation of ligands through use of free lipids, given that the clusters can resemble high density receptors that are present within microvilli [30]. Immediately after modification (0 h), streptavidin was primarily localized on the surface of MSCs whereas after 8 h the residual streptavidin was partially internalized.

3.2. Accessibility of lipids integrated within the cell membrane

Fig. 4C shows the temporal stability and accessibility of the biotin on the MSC surface (modified using vesicles) which was

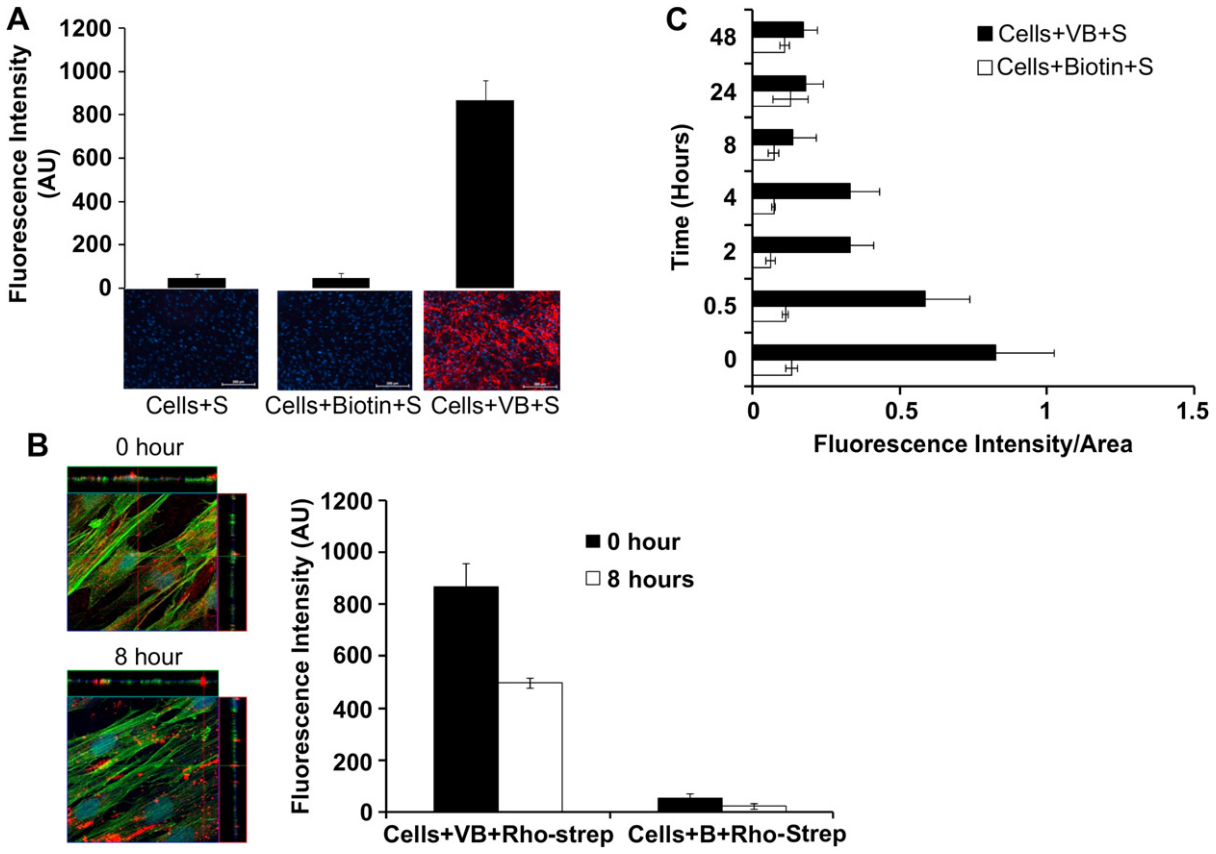

Fig. 4. (A) Modification of the MSCs with biotinylated lipid vesicles (VB) measured as a function of fluorescent signal after addition of Rhodamine–Streptavidin (S). (B) Confocal image of MSCs with biotinylated lipid vesicle and S immediately after modification (0 h) and 8 h after modification. Green-Actin, Blue-Nucleus, Red-Rhodamine–streptavidin conjugated to biotinylated lipid. Stability of streptavidin conjugated to biotin on cell surface measured immediately and 8 h after modification. (C) Accessibility (and stability) of lipid biotin or adsorbed biotin on the MSC surface measured after addition of S at each time point.

examined by quantifying the fluorescence signal of rhodamine–streptavidin added to biotinylated cells at each time point. The presence of fluorescence indicates accessible biotin on the surface of vesicle modified MSCs. MSCs treated with native biotin were considered as negative control. Up to 4 h, the fluorescence intensity of the vesicle modified MSCs was significantly higher than the controls. However, the fluorescence intensity was 50% less compared to the fluorescence intensity observed at 0 h. After 8 h, there was no significant difference in the fluorescence intensity between the vesicle modified MSCs and the controls and the fluorescence was 80% less compared to the fluorescence intensity observed at 0 h. This indicates that biotinylated lipids are not retained on the cell surface which could be due to two reasons: the biotinylated lipids are either internalized within the cell or escaping from cell surface into the external environment. Thus, vesicle mediated biotin-lipid insertion does not permanently modify the cell surface but temporarily allow the conjugation of ligand(s) through the streptavidin–biotin bridge. This short-term stability and accessibility of the biotin on the cell surface potentially permit the cell to home to particular tissue without long-term expression of the modified ligands on the cell surface.

3.3. Examination of modified MSCs under flow conditions

The rolling and adhesive interactions of the SLeX-engineered MSCs with P-selectin coated substrates using a flow chamber assay. Typically, culture expanded MSCs do not express ligands to enable cell rolling on P-selectin, which limits their homing capacity. By contrast, MSCs modified with SLeX showed considerably lower velocities on immobilized P-selectin substrates compared to PBS treated cells (Fig. 5A). SLeX modified MSCs exhibited an average velocity of 8 μm/s at a shear stress of 0.5 dynes/cm^2 whereas unmodified MSCs exhibited an average velocity of 61 μm/s. The average velocity of the MSCs modified with biotinylated lipid vesicles (VB) without streptavidin or SLeX was 31 μm/s and no rolling cells were observed, indicating that the vesicles alone on the MSC surface do not contribute to the rolling observed with the SLeX modified MSCs. To investigate the specificity of the interaction between SLeX and P-selectin, SLeX ligand on the MSC surface was blocked with P-selectin which resulted in an increase of the velocity by fourfold. Additionally, the average velocity of MSC modified with a non-cell rolling ligand, biotinylated glucose, was 42 μm/s and no rolling cells were observed. Together, these data indicate that the reduced velocity of SLeX modified MSCs was due

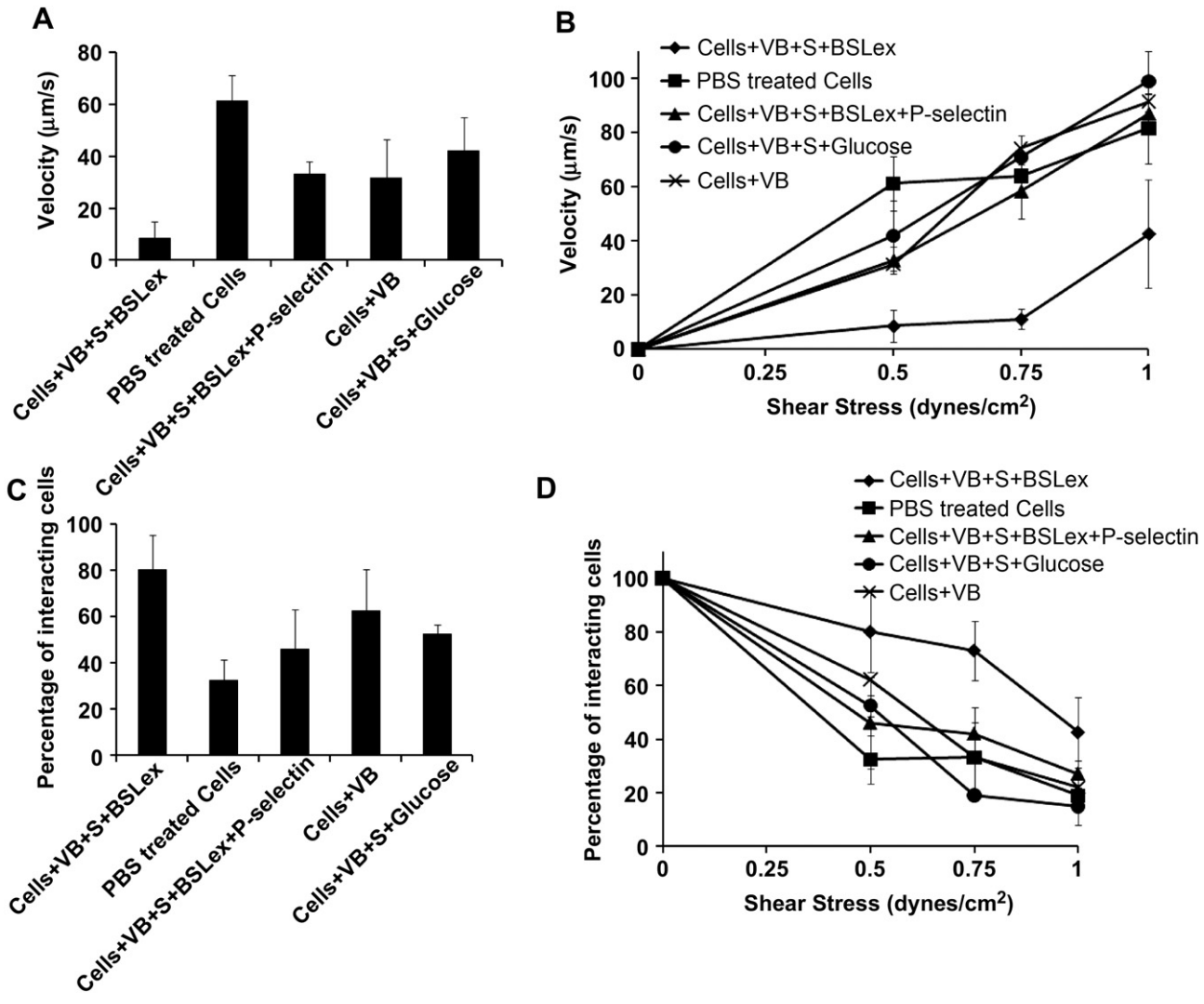

Fig. 5. Flow chamber assay on P-selectin coated surface. (A) Velocity of SLeX modified and PBS treated MSCs at 0.5 dyn/cm^2. (B) Effect of shear stress on the cell velocity. (C) Percentage of cells interacting with the surface for a 10 s period at 0.5 dyn/cm^2. (D) Effect of shear stress on the number of interacting cells.

to specific interaction between SLeX and P-selectin. As shear stress was increased, SLeX modified MSCs exhibited a rolling response up to 0.75 dynes/cm^2 but the velocity increased significantly beyond 1.0 dynes/cm^2 (Fig. 5B). Importantly, the velocity of the SLeX modified MSCs was lower than all the controls at all shear stresses examined. To further characterize the interaction between SLeX functionalized cells and a P-selectin substrate, the flux was calculated by quantifying the total number of interacting cells within field of view for 10 s. Fig. 5C shows that 80% of the SLeX modified MSCs interacted with P-selectin at 0.5 dynes/cm^2 compared to only 32% of unmodified MSCs. The number of interacting cells was lower for all other controls compared to SLeX modified MSCs. At higher shear stress the number of interacting cells decreases with 42% of the SLeX modified cells interacting with P-selectin at 1.0 dynes/cm^2. However, at all shear stresses up to 1.0 dynes/cm^2 the number interacting cells were higher for SLeX modified MSCs compared to all the controls. Both the velocity and the number of interacting cells indicate that SLeX modification of MSCs significantly enhances interaction with P-selectin. This effect is more pronounced at 0.5 dyn/cm^2 as compared to 1 dyn/cm^2. Thus, SLeX modification of MSCs through vesicles shows that the rolling ligands immobilized on cell surface can induce a rolling response and potentially can increase the interaction of MSCs with inflamed vessels to enhance the homing response.

To investigate changes in the rolling response after initial cell surface modification, the rolling interactions of the SLeX modified MSCs at 8 h following modification. After 8 h, the average velocity of the SLeX modified MSC at 0.5 dynes/cm^2 was 8 μm/s which is similar to the velocities of the SLeX-cells at 0 h (Fig. 6A). However, beyond 0.5 dynes/cm^2, the velocity of SLeX-MSC at 8 h increased to 66 μm/s and was similar to the velocities of the MSCs modified with vesicles only. Additionally, after 8 h, 86% of the SLeX modified MSCs were interacting at 0.5 dynes/cm^2 which is comparable to the number of interacting SLeX modified MSCs at 0 h (Fig. 6C). Beyond 0.5 dynes/cm^2, the number interacting of SLeX modified MSCs decreased. Fig. 6B shows that the average velocity of the MSCs modified solely with biotinylated lipid vesicles were similar at 0 h and 8 h after modification. Fig. 6D shows that there is no significant difference in the number of interacting MSCs modified only with biotinylated lipid vesicles at 0 h and 8 h after modification. These results show SLeX immobilized on the surface of the MSCs was at least partially available after 8 h to interact with P-selectin at low shear stress. Overtime the SLeX-engineered MSCs partly lose ligands on cell membrane as observed from the reduced the fluorescence intensity (Fig. 4B) of the rhodamine–streptavidin conjugated to biotinylated cells after 8 h. Additionally, the confocal images (Fig. 4B, 8 h) also show that a fraction of the clustered streptavidin is retained on the MSC surface, while some fraction of streptavidin is internalized. This indicates the short-term stability of the adhesion ligand on the cell membrane to induce adhesive interactions without any permanent modification of the cell.

3.4. Characterization of MSC phenotype

The modification of the MSCs with biotinylated lipid vesicles followed by streptavidin and SLeX did not affect the phenotype of the MSCs. Fig. 7A shows that the viability of MSCs lipid vesicles remains unaffected compared to MSCs treated in PBS. Similar viability between SLeX-MSCs and PBS treated MSCs, immediately after modification (0 h) and 48 h after modification, indicates that the presence of biotinylated SLeX on the MSC surface through biotinylated lipid and streptavidin-bridge does not induce any toxic

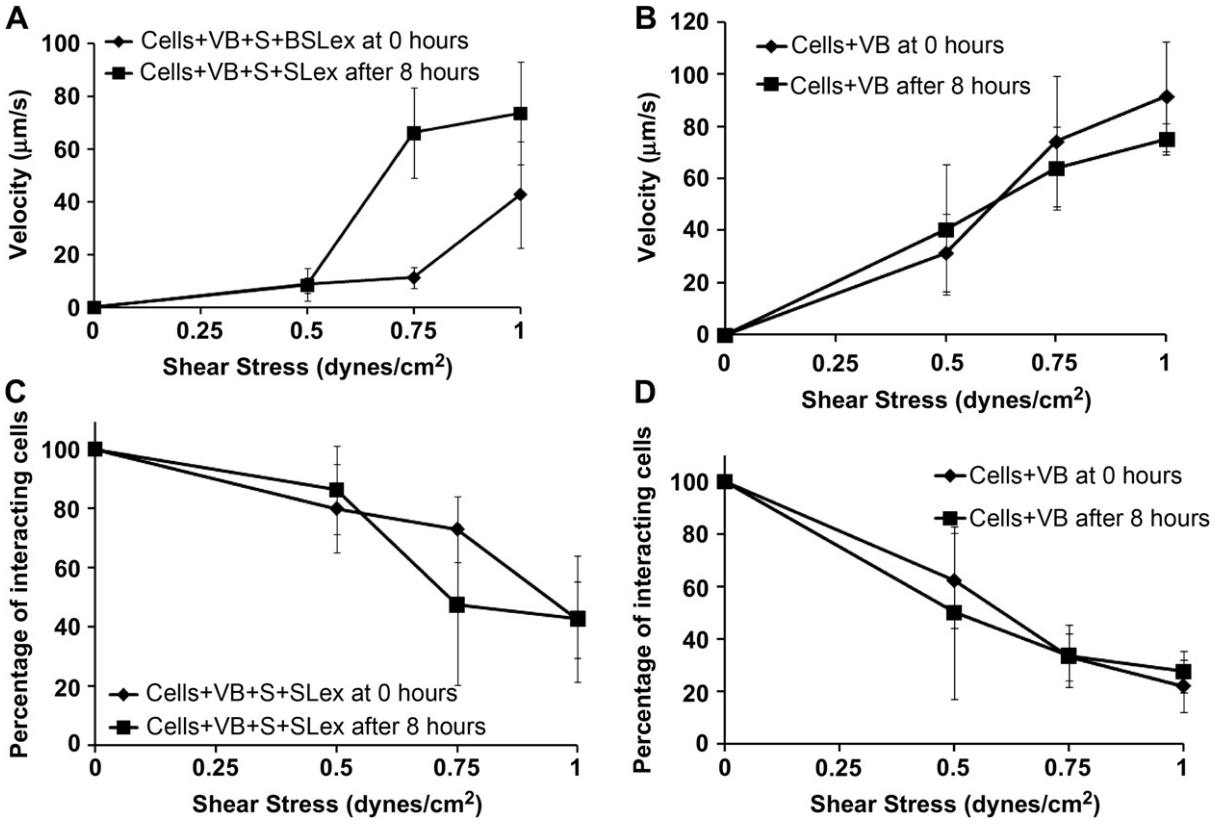

Fig. 6. The stability of rolling interactions 8 h after modification. Velocity of MSCs modified (A) with SLeX and (B) with only biotinylated vesicles immediately and 8 h after modification. Number of interacting MSCs modified with (C) SLeX and (D) only with biotinylated vesicles immediately and 8 h after modification.

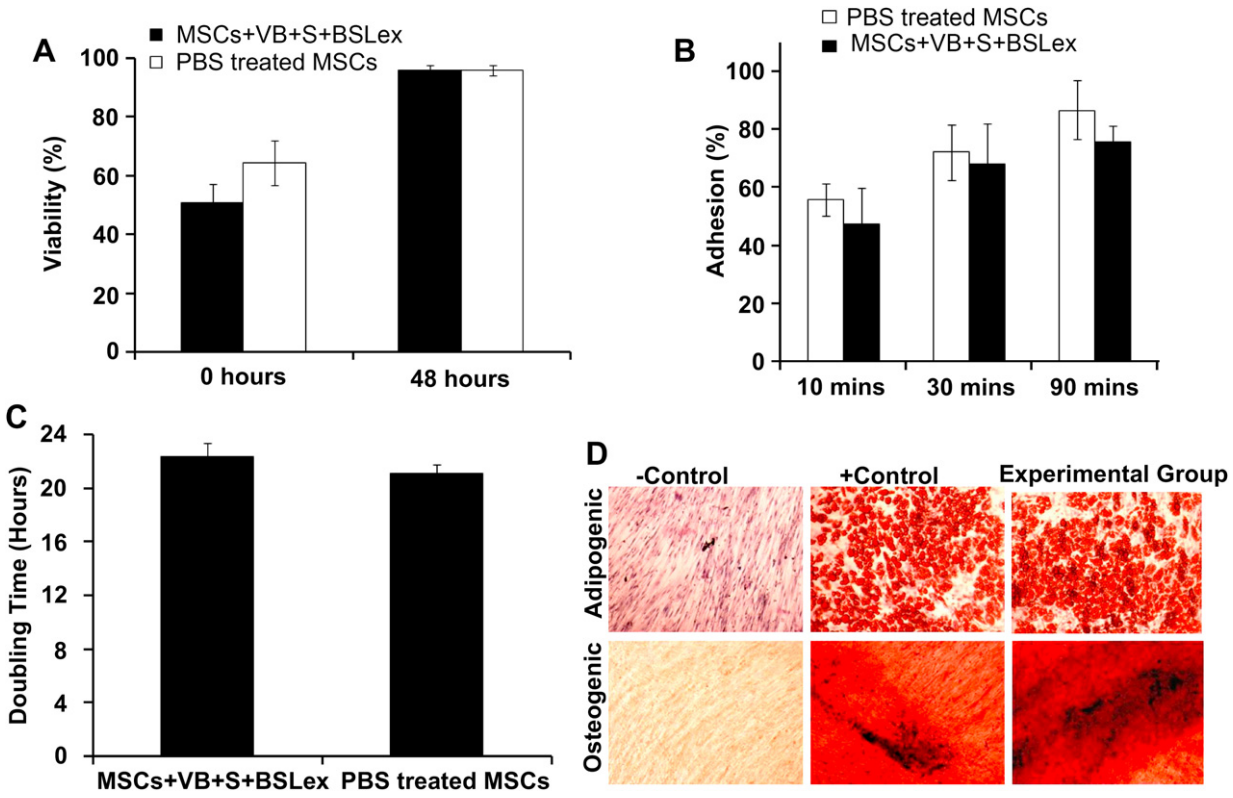

Fig. 7. (A) Viability of the SLeX modified MSCs at 0 h and after 48 h. (B) Adherence of SLeX modified cells compared to the PBS treated cells. (C) Doubling time of the SLeX modified cells compared to PBS treated cells. (D) Alkaline phosphatase and Oil Red O staining, 23 days after addition of osteogenic and adipogenic differentiation media to SLeX modified MSCs. [−Control: SLeX modified MSCs in cell expansion media; +Control: MSCs in differentiation media; Experimental group: SLeX modified MSCs in differentiation media].

effect to cells. Moreover, the adhesion kinetics of modified MSCs and PBS treated MSCs are similar on tissue culture polystyrene surface (Fig. 7B). This indicates the ability of the modified cells to interact with their external environment is not compromised due to the modification. Fig. 7C shows SLeX modified cells and PBS treated hMSCs exhibit comparable rate of proliferation with similar doubling time. This shows that proliferation of SLeX modified MSCs through lipid vesicles do not change the proliferative capacity of the cells. The differentiation potential of the MSCs was retained after SLeX modification: SLeX modified cells were differentiated into osteogenic and adipogenic lineages as shown by alkaline phosphatase (ALP) activity and Oil Red O (ORO) staining, respectively (Fig. 7D). No differences in ORO or ALP staining were observed between modified cells and those treated with PBS after induction of osteogenic and adipogenic differentiation. Modified MSCs cultured in non-differentiating conditions (i.e. in MSC expansion media) did not stain positively for osteogenic and adipogenic markers. Thus, the modification of MSCs with vesicles followed by streptavidin and biotinylated SLeX did not impair the potential for multi-lineage differentiation. The results clearly show that modifying the cells with rolling ligands utilizing a lipid vesicle approach does not affect the cell phenotype and thus cells may maintain their normal functional characteristics which will be important for clinical application.

4. Conclusions

The goal of this study was to test whether the surface of the MSCs can be engineered with self-assembled vesicles to induce a rolling response by mimicking the selectin mediated rolling response of leukocytes. To ensure efficient homing of systemically administered MSCs, it is critical to consider that the engineered MSCs should exhibit rolling interactions with the selectins present on the activated endothelium. These results provide a new alternative to induce leukocyte like rolling characteristics by transiently modifying the MSC surface with self-assembled vesicles. Most importantly, the vesicle modified cells retains their native phenotype i.e. viability, adhesion kinetics, proliferation and multi-lineage differentiation potential of the cells. The SLeX modified MSCs through a biotinylated lipid vesicle approach may potentially be useful to target specific tissues following systemic infusion. More importantly, this methodology presents a generic approach to transiently immobilize ligands on the cell surface using lipid vesicles.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

This work was supported by American Heart Association grant #0970178N and NIH grant #DE019191 to JMK. PKV was supported by Kauffman Foundation Entrepreneur Postdoctoral Fellowship and WZ was supported by Human Frontier Science Program (HFSP) Postdoctoral Fellowship.

Appendix

Figures with essential colour discrimination. Several of the figures in this article have parts that are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2010.03.006.

References

[1] Picinich SC, Mishra PJ, Mishra PJ, Glod J, Banerjee D. The therapeutic potential of mesenchymal stem cells. Cell- & tissue-based therapy. Expert Opin Biol Ther 2007;7:965–73.

[2] Martens TP, See F, Schuster MD, Sondermeijer HP, Hefti MM, Zannettino A, et al. Mesenchymal lineage precursor cells induce vascular network formation in ischemic myocardium. Nat Clin Pract Cardiovasc Med 2006;3(Suppl. 1): S18–22.

[3] Uccelli A, Pistoia V, Moretta L. Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol 2007;28:219–26.

[4] Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008;8:726–36.

[5] Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol 2009;217:318–24.

[6] Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair – current views. Stem Cells 2007;25:2896–902.

[7] Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 2009;4:206–16.

[8] Muschler GF, Nakamoto C, Griffith LG. Engineering principles of clinical cell-based tissue engineering. J Bone Joint Surg Am 2004;86-A:1541–58.

[9] Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infracted myocardium: feasibility, cell migration, and body distribution. Circulation 2003;108:863–8.

[10] Jones EA, Kinsey SE, English A, Jones RA, Straszynski L, Meredith DM, et al. Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. Arthritis Rheum 2002;46:3349–60.

[11] Rombouts WJ, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 2003;17:160–70.

[12] Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 2007;7:678–89.

[13] von Andrian UH, Arfors KE. Neutrophil–endothelial cell interactions in vivo: a chain of events characterized by distinct molecular mechanisms. Agents Actions Suppl 1993;41:153–64.

[14] Smith CW. Possible steps involved in the transition to stationary adhesion of rolling neutrophils: a brief review. Microcirculation 2000;7:385–94.

[15] Bhatia SK, King MR, Hammer DA. The state diagram for cell adhesion mediated by two receptors. Biophys J 2003;84:2671–90.

[16] Cheng Z, Ou L, Zhou X, Li F, Jia X, Zhang Y, et al. Targeted migration of mesenchymal stem cells modified with *CXCR4* gene to infracted myocardium improves cardiac performance. Mol Ther 2008;16:571–9.

[17] Kumar S, Ponnazhagan S. Bone homing of mesenchymal stem cells by ectopic alpha 4 integrin expression. FASEB J 2007;21:3917–27.

[18] Brenner S, Whiting-Theobald N, Kawai T, Linton GF, Rudikoff AG, Choi U, et al. CXCR4-transgene expression significantly improves marrow engraftment of cultured hematopoietic stem cells. Stem Cells 2004;22:1128–33.

[19] Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, et al. Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. Nat Med 2008;14:181–7.

[20] Ko IK, Kean TJ, Dennis JE. Targeting mesenchymal stem cells to activated endothelial cells. Biomaterials 2009;30:3702–10.

[21] Lee RJ, Fang Q, Davol PA, Gu Y, Sievers RE, Grabert RC, et al. Antibody targeting of stem cells to infracted myocardium. Stem Cells 2007;25:712–7.

[22] Blanks JE, Moll T, Eytner R, Vestweber D. Stimulation of P-selectin glycoprotein ligand-1 on mouse neutrophils activates beta 2-integrin mediated cell attachment to ICAM-1. Eur J Immunol 1998;28:433–43.

[23] Eniola AO, Willcox PJ, Hammer DA. Interplay between rolling and firm adhesion elucidated with a cell-free system engineered with two distinct receptor–ligand pairs. Biophys J 2003;85:2720–31.

[24] Sarkar D, Vemula PK, Teo GS, Spelke D, Karnik R, Wee le Y, et al. Chemical engineering of mesenchymal stem cells to induce a cell rolling response. Bioconjug Chem 2008;19:2105–9.

[25] Bajaj A, Kondaiah P, Bhattacharya S. Synthesis and gene transfection efficacies of PEI–cholesterol-based lipopolymers. Bioconjug Chem 2008;19:1640–51.

[26] Verhage M, Sorensen JB. Vesicle docking in regulated exocytosis. Traffic 2008;9:1414–24.

[27] Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997;387:569–72.

[28] Ratanabanangkoon P, Gropper M, Merkel R, Sackmann E, Gast AP. Mechanics of streptavidin-coated giant lipid bilayer vesicles: a Micropipet Study. Langmuir 2003;19:1054–62.

[29] Tsien RY. Fluorescent probes of cell signaling. Annu Rev Neurosci 1989;12: 227–53.

[30] Park EY, Smith MJ, Stropp ES, Snapp KR, DiVietro JA, Walker WF, et al. Comparison of PSGL-1 microbead and neutrophil rolling: microvillus elongation stabilizes P-selectin bond clusters. Biophys J 2002;82:1835–47.