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Cell Surface Conjugation of Sialyl Lewis X Induces a Rolling Response for Mesenchymal Stem Cells

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Abstract- There has been significant interest in the clinical use of adult mesenchymal stem cells (MSCs), which are connective tissue progenitor cells. One of the greatest challenges in traditional stem cell therapy is to deliver a large quantity of viable stem cells with high engraftment efficiency. MSCs home at a low efficiency due to the lack of relevant adhesion molecules on their surface. We have engineered the surface of the MSCs with the sialyl Lewis^x (SLeX) moiety, found on the surfaces of leukocytes representing the active site of the P-selectin glycoprotein ligand (PSGL-1) for inducing rolling response as the first step in the homing process which involves reversible, adhesive interactions between glycoprotein receptors on specific circulating cells and ligands expressed on the surface of the vascular endothelium. MSCs were covalently modified SLeX through biotin-streptavidin linkage and the rolling response of the modified MSCs were examined on P-selectin surface. Modified MSCs exhibited velocities of 2 μ m/sec whereas the unmodified MSCs exhibited velocities of 65 μ m/sec at a wall shear stress of 0.366 dynes/cm² on P-selectin surface in a parallel plate flow chamber assay. Most importantly, the MSCs' native phenotype including its ability to proliferate and differentiate into multi-lineages was retained after the modification. This platform strategy demonstrates the potential to target MSCs to specific tissues within the body by conjugation of specific targeting ligands.

I. INTRODUCTION

Stem cell based therapies offer enormous hope for patients suffering from a wide range of diseases and disorders. Adult mesenchymal stem cells (MSCs), which are connective tissue progenitor cells, are currently in pre-clinical and clinical trials to treat different diseases [1]. It is believed that systemically infused MSCs have the capability to home and engraft followed by in situ proliferation, differentiation, promotion of vascularization, and trophic activity but MSCs home at a low efficiency; typically less than 1% of the infused cells reach the targeted site[2]. Since the therapeutic potential of MSCs depends on their local rate of engraftment, efforts have been made to modify MSCs to increase their homing efficiency through enzymatic [3] and genetic modifications [4] but the efficacy of these methods are limited due to the complexity and the potential safety concerns. Here, we report a simple platform chemical approach to modify MSC surfaces to induce a homing response. The modification consists of 3 steps including: 1. Covalent immobilization of biotin onto the cell membrane, 2. Functionalization of the biotin with streptavidin, 3. Attachment of biotinylated sialyl Lewis X

(SLeX). It is believed that the absence of targeting agents such as SLeX on the surface of MSCs is responsible for their poor homing characteristics [3]. Thus we anticipated that synthetic immobilization of SLeX on the cell surface would induce a homing response through promoting cell rolling as shown in Fig. 1A.

II. MATERIALS AND METHODS

The free amine groups present on the surface of cells reacted with the *N*-hydroxy-succinimide group of sulfonated biotinyl-*N*-hydroxy-succinimide (sulfo-NHS-Biotin, BNHS). 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 biotin-streptavidin complexes. The strong interaction between biotin and streptavidin stabilized the streptavidin on the cell

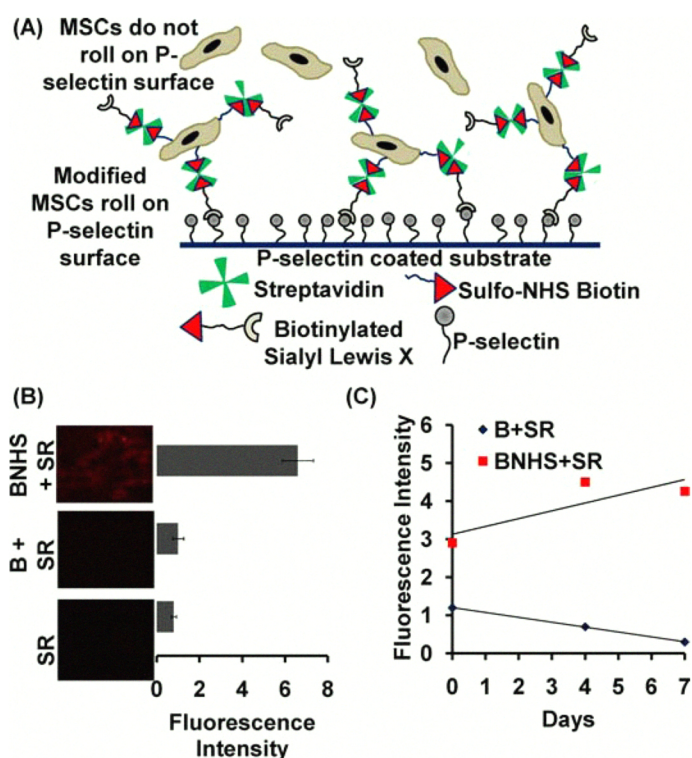


Figure 1.(A) Schematic presentation of rolling of MSCs modified with SLeX through biotin-streptavidin on P-selectin surface (B) Modification of the MSCs measured as a function of streptavidin rhodamine fluorescent signal (C) Stability and accessibility of covalently conjugated or adsorbed biotin on the MSC surface measured by addition of SR at each time point.

surface. The streptavidin was then complexed with biotinylated-SLeX to introduce SLeX on the cell surface. To assess the cell modification by BNHS and streptavidin, rhodamine-conjugated streptavidin (SR) was added to biotinylated cells (BNHS+SR) and the rhodamine fluorescence intensity was measured. The controls used for this experiment were cells treated with only SR and cells treated with biotin (B) and SR (B+SR). The temporal stability and accessibility of biotin on the cell surface was also analyzed by adding rhodamine-streptavidin at 3 time points to the biotinylated cells, and following multiple washes, the fluorescence intensity was measured. The effect of covalent modification on cell characteristics such as viability, proliferation, adhesion, and ability to differentiate into multiple lineages, were examined by a series of experiments. The rolling assays were performed in rectangular parallel plate flow chamber on P-selectin coated substrate with defined shear stress.

III. RESULTS AND DISCUSSION

Cells functionalized with BNHS (covalent attachment) had considerably higher fluorescence intensity compared to cells treated with B+SR and SR (Fig. 1B). This indicates that cells that were covalently functionalized using the NHS moiety have higher amounts of streptavidin immobilized on their surfaces. The fluorescence intensity of rhodamine-streptavidin at 3 time points indicates that MSCs covalently modified with BNHS have significantly higher intensity than MSCs treated with B+SR indicating the stability and accessibility of biotin on the modified MSCs. The characterization of cell phenotype shows that modified MSCs are 80% viable compared to 95% viability unmodified MSCs (Fig 2A). The proliferation (Fig. 2B) and adhesion dynamics (on tissue culture plate, Fig. 2C) of MSCs are not affected due to the modification of the cells. BNHS modified cells were differentiated into osteogenic and adipogenic lineages as shown by alkaline phosphatase (ALP) activity and Oil Red O (ORO) staining, respectively. No differences in ORO or ALP staining were observed between

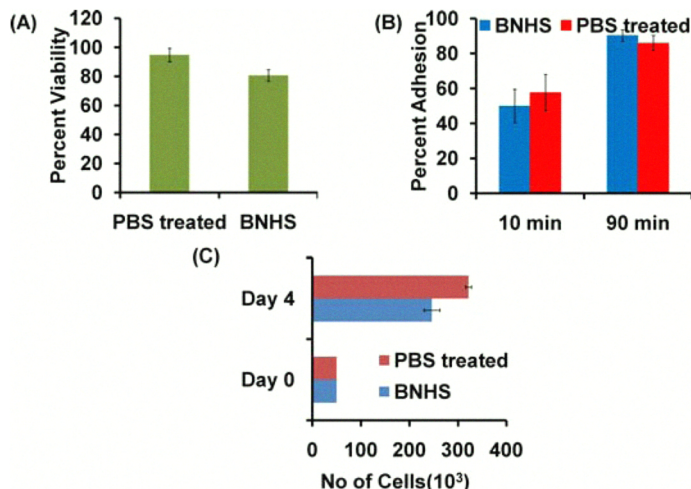


Figure 2. (A) Viability of BNHS modified cells after 0 hours. (B) Adherence of BNHS modified cells measured at 10 and 90 minutes compared to the PBS treated cells. (C) Proliferation of the BNHS modified cells after 4 days compared to PBS treated cells.

BNHS modified cells and PBS treated cells after induction of osteogenic and adipogenic differentiation (results not shown). MSCs modified with BNHS, streptavidin (S) and biotinylated SLeX showed considerably lower velocities on immobilized P-selectin substrates compared to PBS treated cells. The interaction between SLeX and P-selectin reduced the velocity from $\sim 65 \mu\text{m/s}$ to $\sim 2 \mu\text{m/s}$ at a wall shear stress 0.366 dynes/cm^2 (Fig. 3A). Moreover, the flux value of the modified MSCs was 75 cells/mm^2 whereas the unmodified cells displayed a value of 20 cells/mm^2 (Fig. 3B). This clearly indicates that covalent modification of MSCs by biotin-streptavidin followed by SLeX-biotin induces rolling characteristics of culture expanded MSCs that are not typically observed.

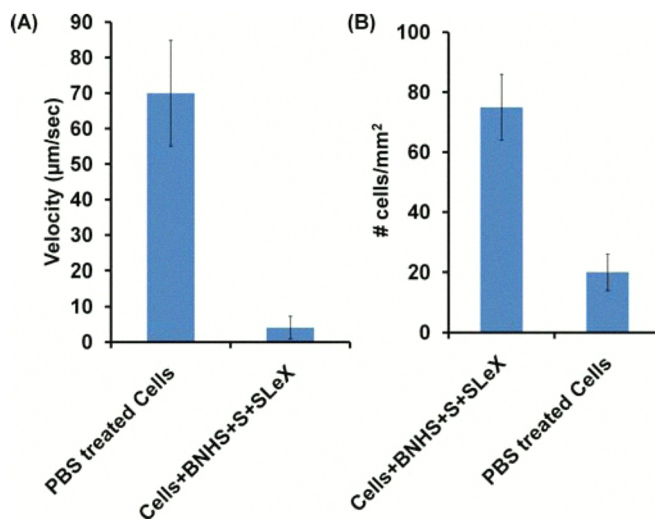


Figure 3. (A) Velocity and (B) Flux of SLeX modified MSCs and controls on P-selectin treated surfaces at the shear stress of 0.366 dynes/cm^2 .

IV. CONCLUSIONS

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.

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