Surface Engineered Mesenchymal Stem Cells for Systemic Cell Targeting

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Statement of Purpose: There has been significant interest in the clinical use of adult mesenchymal stem cells (MSCs) for treating patients suffering from a wide range of diseases[1]. Yet, one of the greatest challenges is to deliver a large quantity of viable stem cells with high engraftment efficiency[1]. The predominant reason for low engraftment efficiency following systemic administration of MSCs is due to the lack of relevant adhesion molecules on their surface following culture expansion[1,2]. To engineer a mesenchymal stem cell homing response, we employed a simple chemical approach. Specifically, the sialyl Lewis^x (SLeX) moiety, found on the surfaces of leukocytes representing the active site of the P-selectin glycoprotein ligand (PSGL-1), was covalently immobilized to the cell surface by biotin-streptavidin conjugation to improve the homing response[3].

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 surface. The streptavidin was then complexed with biotinylated-SLeX to introduce SLeX on the cell surface. In vivo rolling assays were performed in rectangular parallel plate flow chamber on P-selectin coated substrate with defined shear stress to examine the interaction of SLeX modified MSCs under dynamic shear stress condition. The homing efficiency of systemically infused SLeX modified MSCs was examined in LPS induced mouse ear inflammation model.

Results: The adhesive interactions of SLeX modified MSCs investigated under dynamic shear stress condition on P-selectin coated substrate showed 96% reduction of velocity compared to unmodified MSCs. SLeX modified MSCs exhibited velocities of 0.5 μm/sec at a shear stress of 0.5 dynes/cm^2 compared to 70 μm/sec for the unmodified MSCs on P-selectin surface in a parallel plate flow chamber assay (Figure 1A). Most importantly, SLeX modified MSCs exhibited velocities of 2 μm/sec up to a wall shear stress of 1.89 dynes/cm^2. The lower velocity of MSCs modified covalently with SLeX induces a robust rolling response over a physiologically relevant range of shear stresses for efficient homing of systemically delivered MSCs. The homing efficiency of systemically infused SLeX modified MSCs examined in LPS induced

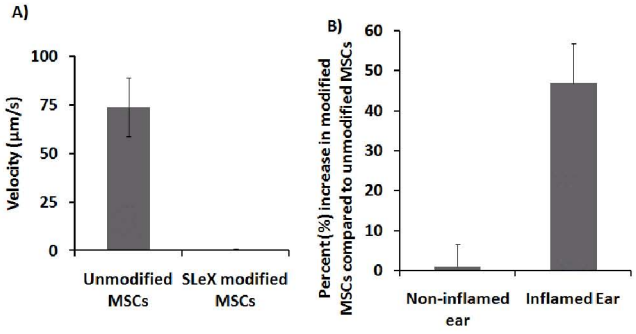

Figure 1 (A) Velocity of un-modified MSCs and SLeX-modified MSCs controls on P-selectin treated surfaces at the shear stress of 0.366 dynes/cm^2 (B) Percentage increase in SLeX modified MSCs to unmodified MSCs that homed into the tissue in the inflamed ear and in the non-inflamed ear after 24 hours of systemic infusion of cells.

mouse ear inflammation model showed 46% increase in the number of SLeX modified MSCs within the inflamed ear at 24hr compared to the unmodified MSCs (Figure 1B) whereas there was no increase in the number of SLeX-modified MSCs the non-inflamed ear. The increased number of engineered cells in the inflamed ear indicates that modified MSCs have increased homing efficiency. Through replacing SLeX with glucose and observing no enhancement in the homing response, the increased homing of SLeX engineered MSCs is likely due to the specific interaction between immobilized SLeX and the selectins upregulated during inflammation. Most importantly, the cell surface modification did not affect the cell phenotype such as viability, proliferation, adhesion, secretion of paracrine factors and ability to differentiate into multiple lineages. This shows that surface engineering of MSCs can be used to increase the homing efficiency of the systemically delivered cells to specific tissues without altering the cell phenotype.

Conclusions: In conclusion, these results show that MSCs covalently conjugated with SLeX can potentially be targeted to inflammatory sites where the normal characteristics of the cells are retained. The approach described here may offer a simple method to target any cell type to specific tissues within the body.

References:

[1] Karp JM. Cell Stem Cell. 2009; 4:206-216
[2] Sackstein R. Nat Med. 2008; 14: 181-187
[3] Sarkar D. Bioconjugate Chem. 2008; 19: 2105-2109