Published in final edited form as:

Biomaterials. 2016 January ; 74: 19–30. doi:10.1016/j.biomaterials.2015.09.026.

Cell surface glycoengineering improves selectin-mediated adhesion of mesenchymal stem cells (MSCs) and cardiosphere-derived cells (CDCs): pilot validation in porcine ischemia-reperfusion model

Chi Y. Lo^{1,2,4}, Brian R. Weil⁴, Beth A. Palka⁴, Arezoo Momeni¹, John M. Canty Jr.^{4,5}, and Sriram Neelamegham^{1,3,*}

¹Department of Chemical and Biological Engineering, The State University of New York, 906 Furnas Hall, Buffalo, NY 14260, USA

²Department of Anesthesiology, The State University of New York, 252 Farber Hall, Buffalo, NY 14214, USA

³The NY State Center for Excellence in Bioinformatics and Life Sciences, The State University of New York, 701 Ellicott St., Buffalo, NY 14203, USA

⁴Division of Cardiovascular Medicine, The State University of New York, Clinical Translational Research Center, 875 Ellicott Street, Buffalo, NY 14203, USA

⁵VA Western New York Health Care System, Buffalo, NY 14215, U.S.A

Abstract

Promising results are emerging in clinical trials focused on stem cell therapy for cardiology applications. However, the low homing and engraftment of the injected cells to target tissue continues to be a problem. Cellular glycoengineering can address this limitation by enabling the targeting of stem cells to sites of vascular injury/inflammation. Two such glycoengineering methods are presented here: i. The non-covalent incorporation of a P-selectin glycoprotein ligand-1 (PSGL-1) mimetic 19Fc[FUT7⁺] via lipid-protein G fusion intermediates that intercalate onto the cell surface, and ii. Over-expression of the $\alpha(1,3)$ fucosyltransferase FUT7 in cells. Results demonstrate the efficient coupling of 19Fc[FUT7⁺] onto both cardiosphere-derived cells (CDCs) and mesenchymal stem cells (MSCs), with coupling being more efficient when using protein G fused to single-tailed palmitic acid rather than double-tailed DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine). This non-covalent cellular modification was mild since cell proliferation and stem-cell marker expression was unaltered. Whereas coupling using 19Fc[FUT7⁺] enhanced cell capture on recombinant P-selectin or CHO-P cell surfaces, $\alpha(1,3)$ fucosylation was necessary for robust binding to E-selectin and inflamed endothelial cells under shear. Pilot studies confirm

*Corresponding Author. 906 Furnas Hall, State University of New York, Buffalo, NY 14260, USA; Tel.: +1 716 645 1200; Fax: +1 716 645 3822. neel@buffalo.edu (S. Neelamegham).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the safety and homing efficacy of the modified stem cells to sites of ischemia-reperfusion in the porcine heart. Overall, glycoengineering with physiological selectin-ligands may enhance stem cell engraftment.

Keywords

mesenchymal stem cells; adhesion molecule; blood flow; cell adhesion; recombinant protein; selectin; PSGL-1; sialyl Lewis-X

1. Introduction

Both mesenchymal stem cells (MSCs) and cardiosphere-derived cells (CDCs) are candidate cell types currently being tested in early phase clinical trials to determine their efficacy in treating various cardiovascular ailments [1-4]. Among these, MSCs are commonly used since they can be easily isolated from a bone marrow aspirate or adipose tissue [5]. The CDCs are a relatively newer stem cell type that is expanded from myocardial tissue biopsies [6]. Both these cells have regenerative potential and are considered immunologically privileged; thus, allogeneic transplantation is feasible. While both the MSCs and CDCs can differentiate into cardiomyocytes, the efficiency of this process *in vivo* may be low [6-9]. Thus, rather than the transplanted stem cells themselves replenishing myocytes, secreted paracrine material from the transplanted cells (e.g., growth factors, micro RNAs and exosomes) may promote endogenous myocyte proliferation [10]. Besides paracrine effects, cell-cell contact may also contribute to the observed beneficial effects of stem cell therapy [11]. Regardless of the repair mechanism, studies have shown that increased cellular engraftment directly correlates with efficacy and functional outcomes [12, 13]. Therefore, there is currently considerable interest to develop methods for the efficient delivery of stem cells for regenerative therapy.

The two most common modes of stem cell delivery to the heart employ either direct injection into the cardiac muscle or vascular infusion, either into the coronary or venous circulation [14]. Neither approach results in substantial stem cell retention in the heart tissue with >90% of the injected cells no longer present 24h following treatment [14]. While intra-myocardial injection leads to very precise tissue targeting, the damaged or infarcted tissue itself may be poorly perfused which compromises cell viability [15]. Direct infusion into blood is less invasive and has the advantage that it can be combined with other procedures like percutaneous coronary interventions. Thus, multiple stem cell treatments to the same patient are feasible via this route. A majority of studies that practice intracoronary infusion employ the 'stop flow' technique, where the coronary vessel is transiently occluded proximal to the target tissue [16, 17]. In principle, such flow stoppage allows time for the stem cells to adhere to the vascular wall. A systematic comparison of this balloon occlusion method with direct infusion without stop-flow, however, demonstrates no difference in cell retention between the two methods at 24h following cell delivery [18]. This could be because the stop-flow method does not take advantage of the rheological properties of flowing blood which marginate the less deformable cell types towards the vessel wall [19].

In recent work, we applied global intracoronary infusion (without stop-flow) to deliver MSCs and CDCs to the porcine hibernating myocardium [20, 21]. The infused cells were clearly observed in the interstitial space, surrounded by endogenous myocytes [21]. Whereas improved myocardial function was noted at 2-4 weeks following CDC infusion in terms of increased regional anterior wall thickening, left ventricular ejection fraction and myocyte regeneration, only ~3% of the infused cells were present in the heart [21]. With the goal of improving cell retention, the current manuscript evaluated two strategies to improve cardiac relevant stem cell targeting, by modifying the MSCs and CDCs with functional carbohydrate-ligands that can bind selectins expressed on the coronary vessel wall at sites of injury [22-24]. First, 19Fc[FUT7⁺] was non-covalently immobilized on CDCs/MSCs. This fusion protein contains the first 19 N-terminal amino acids of human P-selectin glycoprotein ligand-1 (PSGL-1) along with a human IgG1 C-terminus that binds lipidated protein G intercalated into the stem cell membrane. Due to its production in HEK293T cells that expressing the $\alpha(1,3)$ fucosyltransferase FUT7, 19Fc[FUT7⁺] is decorated by a core-2 sialyl Lewis-X selectin-ligand at its N-terminus [25, 26]. Second, the FUT7 enzyme itself was overexpressed on MSCs/CDCs to fucosylate endogenous proteins on the stem cell surface [27, 28]. These optimization studies are necessary since the glycoprotein and lipid compositions of different stem cell types may vary. Thus both the pattern of fucosylation and lipid incorporation may vary with stem cell type, plasma membrane composition and cell size. The effect of surface modification on the underlying cell phenotype and selectin-dependent cell adhesion under fluid shear was quantified. *In vivo* studies in a porcine ischemia-reperfusion model confirm that the modified cells are safe over a 4h time course and that they are retained at sites of injury.

2. Materials and Methods

2.1 Cell culture and stem cell isolation

MSCs and CDCs were isolated from healthy swine as previously described [20, 21]. Procedures and protocols conformed to institutional guidelines for the care and use of animals in research. Briefly, for MSC isolation, 30 ml of bone marrow from the sternum of propofol sedated swine was added to a BD Vacutainer CPT (Cell Preparation Tube) with sodium heparin (12 U/ml final) and Ficoll-Hypaque density fluid (Becton Dickinson, San Jose, CA). After centrifugation at 1160×g for 20 min at RT (room temperature), ~2 ml of the mononuclear cell layer was removed from the buffy coat layer and washed twice using Hanks Balanced Salt Solution (HBSS). Cells were plated and cultured in ADMEM (Advanced DMEM medium, Life Technologies, Grand Island, NY) with 10% FBS. Non-adherent cells were removed by washing plates at 2 and 4 days following plating. The remaining cells were called MSCs. For CDC isolation, left ventricular tissue biopsies were taken from the heart and placed immediately in cold PBS. Tissue was minced into 1 mm pieces and washed twice with PBS. Warm 1 mg/ml collagenase type 2 (Worthington Biochemical Corporation, Lakewood, NJ) dissolved in DMEM/F12 (HyClone, Logan, UT) was added to the pieces for 1 h at 37°C. Tissue was washed with CEM (Complete Explant Media – Iscove's Modified Dulbecco's Media, 20% FBS, 1× GlutaMAX, 1× Pen Strep, and 100 nM β -mercaptoethanol) and plated with CEM onto fibronectin coated plates. After 1 week, cells were trypsinized and plated onto low attachment plates for cardiosphere

formation. After another week, cardiospheres were plated onto fibronectin plates to yield CDCs. The average cell diameter of MSCs and CDCs in suspension, determined by an automatic cell counter (Bio-Rad), was $16.3 \pm 4.7 \mu\text{m}$ and $18.2 \pm 5.1 \mu\text{m}$ (mean $\pm$ SD), respectively (Supplemental Fig. 1).

2.2 Over-expression of FUT7 in stem cells

FUT7 was overexpressed in stem cells in the form of a FUT7-DsRed fusion protein. To create this, full length human FUT7 cDNA available from a previous study [29] was PCR amplified without the stop codon. This was ligated into the lentiviral pCSCG vector that contained DsRED to obtain 'pCSCG-FUT7-DsRed' [26]. High-titer lentivirus was generated using this plasmid and these were used to stably transduce both the MSCs and CDCs. The resultant cells are called 'MSC-FUT7' and 'CDC-FUT7'.

2.3 Lipid conjugation to protein G

Palmitic acid N-hydroxysuccinimide ester (NHS-palmitate) was purchased from Sigma (St. Louis, MO). 18:1 Dodecanyl DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(dodecanyl)) was purchased from Avanti Polar Lipids (Alabaster, AL). Sulfo-NHS and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were dissolved in 4% ethanol in water. Fifty molar excess of sulfo-NHS and EDC were added to DOPE and this mixture was left rotating overnight at RT. The reacted lipid was then extracted 3-times using chloroform with excess liquid being evaporated using a rotary evaporator. Recombinant protein G (Thermo-Pierce) dissolved in PBS (pH 7.8) with 0.3% sodium deoxycholate was coupled to activated lipids (NHS-palmitate and NHS-DOPE) by mixing 10 $\times$ molar excess of lipid with protein G for 20 h at 37°C. Reaction products, palmitate-protein G (PPG) and dodecanyl DOPE-protein G (DPG) conjugate were purified using a PD-10 desalting column (GE Healthcare, Piscataway, NJ) using PBS (pH 7.8) with 0.1% sodium deoxycholate as the elution buffer. The product was passed through a 0.45 μm cut-off membrane filter. Protein concentration was determined using a Coomassie Protein Assay Kit (Thermo). The buffer was exchanged to PBS with a Zeba Desalting column (Thermo) just prior to use.

2.4 Coupling 19Fc/19Fc[FUT7⁺] to cell surfaces

Both 19Fc and 19Fc[FUT7⁺] contain the first 19 amino acids of native PSGL-1 fused to a human IgG1 tail [25]. 19Fc was expressed in wild-type HEK293T cells while 19Fc[FUT7⁺] was expressed in HEK293T cells that overexpress the $\alpha(1,3)$ fucosyltransferase FUT7. Due to this, while 19Fc has a core-2 glycan with an extended sialyl lactosamine structure, 19Fc[FUT7⁺] is decorated with a core-2 sialyl Lewis-X (sLe^X) O-glycan [25]. The sLe^X O-glycan is necessary for selectin recognition, and thus 19Fc[FUT7⁺] binds selectins while 19Fc does not. For coupling these proteins to cells, both normal and FUT7 expressing MSCs or CDCs were trypsinized, washed and resuspended at 10^7 cells/mL in HEPES buffer (30 mM HEPES, 110 mM NaCl, 10 mM glucose, 10 mM KCl, 1 mM MgCl₂, pH = 7.2). PPG/DPG was added to cells at varying concentrations, temperatures and incubation times. These lipidated proteins spontaneously intercalate with the stem cell membrane. Protein G conjugated cells obtained in this manner were washed with HEPES buffer, incubated with purified 19Fc/19Fc[FUT7⁺] for 30 min at RT, and then washed again prior to experimentation. Unless otherwise mentioned, all studies used the 'standard coupling

conditions', defined as 400 µg/ml PPG/DPG for 1 h at 37°C, followed by 30 min incubation with 200 µg/ml 19Fc/19Fc[FUT7⁺] for 30 min at RT. For the time course study, cells were replated following surface-functionalization onto tissue culture plates and removed with 5 mM EDTA in PBS at the indicated time points for flow cytometry analysis.

2.5 Flow cytometry

2×10⁶ cells/mL resuspended in HEPES buffer were incubated with FITC conjugated goat anti-human (GaH) IgG Ab (H+L) [F(ab')₂] (Jackson ImmunoResearch, West Grove, PA), FITC conjugated AAL (*Aleuria Aurantia lectin*) which binds α1,3/6fucose (Vector Labs, Burlingame, CA), APC conjugated anti-CD90/Thy1 clone 5E10 (Biolegend, San Diego, CA) or FITC conjugated anti-CD105 clone MEM-229 (Abcam, Cambridge, MA) at RT for 20 min. Samples were then washed prior to analysis using either an Accuri C6 (BD Biosciences) or a FACSCalibur (BD Biosciences) flow cytometer. In the case of the mouse anti-CD15s/sLe^X mAb CSLEX-1, the unconjugated mAb was initially incubated for 20 min with cells, prior to washing and addition of a secondary Alexa-488 conjugated mAb for 20 more min prior to flow cytometry detection.

2.6 Confocal microscopy

Cells were coupled with PPG/DPG and 19Fc as described above. Following this, the cells were washed and incubated with 20 µg/ml FITC conjugated goat anti-human IgG Ab (H+L) [F(ab')₂] for 20 min at RT, washed twice using HEPES buffer, fixed using HEPES containing 0.5% paraformaldehyde, pelleted, resuspended with ProLong Gold (Life Technologies) and mounted on microscope slides. Samples were imaged using a Zeiss LSM 710 AxioObserver confocal microscope (Jena, Germany) with a Plan-Apochromat 40×/1.3 Oil DIC M27 objective. All images were acquired under identical exposure conditions.

2.7 BrdU proliferation assay

12-15,000 CDCs or MSCs/cm² were plated in T-25 flasks either on the day of PSGL-1 fusion protein coupling or 6 days thereafter. One day after plating, 10 µM BrdU was added for an additional 24-48h. Following this, BrdU cultured cells were trypsinized and treated with 95% ethanol-5% acetic acid for 30 min on ice. These cells were then permeabilized with 0.5% triton-X in PBS for 10 min at RT, unmasked with 2N HCl for 12 min at RT, blocked with 1% BSA in PBS for 10 min at RT, and then finally stained with Alexa Fluor 488 (AF488) conjugated anti-BrdU antibody clone MoBU-1 (Life Technologies) for 30 min at RT. Cells were washed with PBS between each of the above steps. The final sample prepared in this manner was read using a flow cytometer.

2.8 Flow chamber assay

In some cases, recombinant P- or E-selectin Fc fusion protein (R&D Systems, Minneapolis, MN) was spotted on tissue-culture treated petri dishes overnight at 4°C to obtain ~180 sites/µm², as described previously [30]. The entire plate was then blocked with PBS containing 3% bovine serum albumin (BSA). In other cases, monolayers of P-selectin bearing CHO cells (CHO-P) or human umbilical vein endothelial cells (HUVECs) were cultured to confluence on the same petri dish surface. The HUVECs were stimulated with

5U/mL human IL-1 β for 4h prior to experimentation. During individual runs, a custom 400 μ m wide $\times$ 100 μ m high $\times$ 1 cm long rectangular microfluidic flow chamber was vacuum sealed onto the selectin bearing substrate and the entire assembly was placed on a Zeiss Axio Observer microscope. A PCO edge sCMOS camera (PCO AG, Kelheim, Germany) and Zen software (Zeiss) were available for video recording. Cells, coupled with PPG/DPG and 19Fc/19Fc[FUT7⁺], with or without FUT7 overexpression, were perfused in this flow chamber at varying wall shear stresses in HEPES buffer containing 0.1% human serum albumin (HSA) and 1.5 mM Ca²⁺. The number of interacting cells was counted as the sum of adherent and rolling cells at 3 min after the start of cell perfusion. Blocking studies were performed using mouse anti-human IgG monoclonal antibodies (mAbs): anti-PSGL-1/CD162 clone KPL1 (BD Pharmingen, San Jose, CA), anti-P-selectin/CD62P clone G1 (Ancell, Bayport, MN) or anti-E-selectin/CD62E clone P2H3 (eBioscience, San Diego, CA).

2.9 Porcine ischemia-reperfusion model

Healthy female Yorkshire swine (38 $\pm$ 3 kg) were sedated with a Telazol (100 mg/mL)/Xylazine (100 mg/mL) mixture (0.04 mL/kg i.m.), maintained on a continuous intravenous infusion of propofol (5-10 mg/kg/hr), and mechanically ventilated with an oxygen/air mixture at a respiratory rate of $\sim$ 15 breaths/minute throughout the study [21]. Following percutaneous catheterization of a femoral artery, a 6-Fr guiding catheter (Cordis) was advanced into the left main coronary artery under fluoroscopic guidance. Next, an appropriately-sized balloon angioplasty catheter (Boston Scientific, Maverick, 3.0 - 4.0 mm) was advanced and inflated distal to the second angiographically-visible branch of the left anterior descending (LAD) coronary artery for 10 min. To minimize lethal arrhythmias, all animals were pre-treated with Amiodarone (5mg/kg i.v.) and Lidocaine (1.5mg/kg i.v.) and both drugs were continuously infused intravenously (Amiodarone: 0.04mg/kg/min, Lidocaine: 0.05mg/kg/min) during coronary artery occlusion and 10 min into the reperfusion period. Complete cessation of flow during occlusion and full reperfusion after balloon deflation were confirmed by angiography.

Male donor-derived CDCs over-expressing FUT7 were surface modified with 19Fc[FUT7⁺] using PPG as described above. These cells were filtered through a 30-100 μ m pore filter (MACS pre-separation filters, Miltenyi Biotec) to remove any cell aggregates, suspended in heparinized HBSS solution (100U/mL heparin). Thirty min following reperfusion, 8 $\times$ 10⁶ male, modified donor cells were administered via intracoronary infusion under continuous flow through a 6-Fr Sones catheter positioned in the proximal LAD at 10⁶ cells/minute. Echocardiography and electrocardiographic analysis was performed periodically. 3h later, the animal was deeply anesthetized with inhaled isoflurane (4%) and euthanized via KCl administration to the LV chamber. The heart was rapidly excised, rinsed with saline, and the left ventricle was sectioned for post-mortem assessment of modified CDC retention and staining with 1% 2,3,5-triphenyltetrazolium chloride (TTC) for 30 min to exclude myocardial necrosis.

Myocardial retention of modified CDCs was quantified using real-time PCR (CFX Real-Time PCR System, BioRad) and SsoFast EvaGreen Supermix (BioRad) based on published protocols [31]. Briefly, genomic DNA was prepared from modified CDCs, modified CDC-

treated heart tissue, and untreated female porcine heart tissue negative control (Qiagen). DNA amount was quantified using a NanoDrop 2000 spectrophotometer. PCR primers targeted both the porcine Y-chromosome specific SRY (fwd 5' CTCACAGCCCATGAACATAACC 3', rev 5' GAAAGTCCCGGCTGTAAACC 3'), and human FUT7 (fwd 5' AATGAGAGCCGATACCAACG 3', rev 5' TGAAACCAACCCTCAAG GTC 3') genes of the modified cells. A standard curve for FUT7-modified CDCs was generated by serially diluting FUT7-modified CDC genomic DNA from 60,000 to 60 pg, where SRY was the target. A plasmid containing an insert to the FUT7 gene was serially diluted from 100,000 - 10 copies for the quantification of FUT7. All standard curve reactions contained a background of 100 ng untreated female heart genomic DNA. 100 ng of DNA from FUT7-modified CDC treated heart tissue was run per PCR reaction. 5 reactions were run per sample for both SRY and FUT7 detection. Porcine GPI (glucose-6-phosphate isomerase, fwd 5' ATTCAGGACGTTCAACTCAATAGG 3', rev 5' GGGACTATGACTGTC AGGTA AGG 3') served as a positive control for the presence of porcine DNA. Data were processed using BioRad CFX Manager software and included melt-curve analysis for verification of single PCR products.

2.8 Statistics

Data are presented as mean $\pm$ SEM. Student's t-test was performed for dual comparisons. Oneway ANOVA followed by the Tukey-Kramer test was used for multiple comparisons. $P < 0.05$ was considered significant.

3. Results

3.1 19Fc coupling onto MSCs or CDCs

The chemical structures of palmitic acid and dodecanyl DOPE are shown in Fig. 1A. Both lipids were coupled to protein G creating palmitate protein G (PPG) or dodecanyl DOPE protein G (DPG). Simple incubation of PPG or DPG with either MSCs or CDCs, either wild-type cells or those overexpressing FUT7, resulted in the robust display of protein G on the cell surface (Fig. 1B). Further incubation of the cells with recombinant PSGL-1 protein, 19Fc or 19Fc[FUT7⁺], allowed the non-covalent coupling of these fusion proteins to cell-surface protein G. This was confirmed using flow cytometry and a fluorescent antibody against human Fc (Fig. 1C). This antibody bound cells coupled with 19Fc fusion protein at substantially higher levels compared to either the CDCs/MSCs alone or these same cells with protein G conjugated to palmitic acid or DOPE. While experimental data in Fig. 1-4 are presented for 19Fc, similar observations were also made using 19Fc[FUT7⁺] (data not shown). Also, the absolute number of protein G or 19Fc bound to cells was not quantified since fluorescent whole antibodies necessary for such determination would bind with different affinities to cell-surface protein G compared to flow cytometry calibration beads used in such assays.

3.2 Optimization of PPG/DPG incubation

Studies were performed to assess the effect of temperature, time and lipid concentration on the cell surface intercalation of PPG or DPG onto both the CDCs and MSCs (Fig. 2). After

incubation with 19Fc, an anti-human IgG antibody was used to detect the amount of protein on the cell surface.

In terms of temperature, cell surface binding of the PSGL-1 fusion proteins was typically superior at 37°C. Further, coupling using DPG was more temperature sensitive compared to PPG (Fig. 2A, B). In the latter case, the amount of 19Fc bound was equally efficient at both 37°C and room temperature (RT) for both the cell types tested.

In studies that evaluated the effect of lipid incubation time, the incorporation of PPG or DPG could be measured in both the CDCs and MSCs at 15 min, the earliest time point tested (Fig. 2C, D). At later times, greater amounts of 19Fc were detected on the MSCs compared to the CDCs. Further, in the case of CDCs, PPG was more effective at coupling 19Fc to the cell surface compared to DPG at shorter incubation times (15-45 min). The incorporation of PPG or DPG was not statistically different at longer times.

In the final experiments, the concentration of either PPG or DPG was varied from 1-400 µg/ml (Fig. 2E, F). Here, the extent of 19Fc incorporation increased approximately linearly with lipid dose. The amount of 19Fc binding to PPG was somewhat higher compared to DPG, though this difference did not reach statistical significance. Overall, greater amounts of 19Fc were typically detected on MSCs compared to CDCs under identical conditions. Based on the above titration studies, all subsequent sample preparations used 'standard coupling conditions' with 400 µg/ml PPG/DPG added for 1 h at 37°C, followed by 200 µg/ml 19Fc incubation for 30 min at RT except where indicated.

3.3 Lifetime measurements of 19Fc on CDCs and MSCs

Confocal microscopy experiments were performed to confirm the cell surface incorporation of 19Fc onto the CDC/MS cell surface (Fig. 3). Here, following incubation with either PPG or DPG along with 19Fc, the fluorescent anti-human Ab was applied. These studies confirm that 19Fc was indeed localized on the cell surface (Fig. 3A, B).

Flow cytometry measured the lifetime of 19Fc on the cell surface, both in the case of the CDCs and MSCs (Fig. 3C and D). Here, higher fluorescence was measured initially for PPG indicating that this was superior to DPG in terms of 19Fc capture. The MSCs also displayed higher fluorescence compared to the CDCs both for PPG and DPG. Following incorporation, 19Fc levels on cells was reduced over time with ~60-80% of the protein being lost by 24h and no detectable protein measured at 48h.

3.4 Cell surface markers and proliferation of modified CDCs and MSCs

Studies were performed to determine if the CDCs and MSCs treated with PPG/DPG along with 19Fc alter the differentiation status or proliferative ability of these cells (Fig. 4). Such studies measured the percentage of stem cells that expressed progenitor markers CD90 and CD105 either 1-day or 7-days after 19Fc coupling using either PPG or DPG. Additionally, cell proliferation was measured by quantifying BrdU incorporation. In control runs, the protein-coupled lipid and 19Fc were omitted from the cells. Here, CD105 and CD90 expression was not significantly different in any of the samples at either day-1 (Fig. 4A) or

-7 (Fig. 4B). Cell proliferation was also not different between the different treatments. Thus, surface coupling of stem cells does not alter cell antigenic phenotype or proliferation.

3.5 Rolling function of modified CDCs and MSCs

Parallel plate microfluidic flow chamber studies confirmed that the CDCs and MSCs modified with 19Fc[FUT7⁺] displayed enhanced cellular interactions with P-selectin bearing substrates (Fig. 5). Here, 19Fc and 19Fc[FUT7⁺] were coupled onto stem cells using PPG or DPG in order to test their selectin binding ability under fluid flow conditions. Since 19Fc lacks sLe^X at its N-terminal O-glycan, it served as a non-functional, negative control for 19Fc[FUT7⁺].

The results demonstrated that CDCs and MSCs coupled with 19Fc[FUT7⁺] could be captured by P-selectin bearing substrates under continuous fluid shear (Fig. 5A, C). Such binding supported both cell rolling and adhesion for both cell types when 19Fc[FUT7⁺] was surface-coupled but not when the control 19Fc construct was applied. Further, PPG mediated coupling resulted in robust cellular interactions in the entire shear range tested from 2-6 dyne/cm². In contrast, coupling with DPG resulted in less binding at all shear stresses. Consistent with the surface functionalization data, the MSCs also exhibited greater adhesion to P-selectin compared to the CDCs under identical conditions. In all cases, the measured binding was specific since it could be blocked using mAbs against either the PSGL-1 portion of 19Fc[FUT7⁺] (clone KPL1) or anti-P-selectin blocking mAb G1 (Fig. 5B, D). Overall, 19Fc[FUT7⁺] coupling on CDCs/MSCs with PPG showed improved binding to P-selectin compared to DPG under shear.

3.6 Effect of FUT7 over-expression on E-selectin binding

Next, following previous reports [27, 28], we sought to over-expressing the enzyme FUT7 in the stem cells in order to enhance their adhesion to selectin bearing substrates under flow. Such studies were performed with both the MSCs and CDCs, with similar results. Data are thus only provided for the MSCs. Here, FUT7 was over-expressed using a lentivirus that encoded for a FUT7-DsRed fusion protein (Fig. 6A). The transduced cells displayed brilliant red fluorescence (Fig. 6B), with >90% of the cells stably expressing the FUT7 enzyme as determined using flow cytometry (Fig. 6C). These MSC-FUT7 cells displayed elevated AAL binding, a lectin that recognizes α 1,3/6-linked fucose. They also bound CSLEX-1, a mAb recognizing sialyl Lewis-X glycans, at levels higher than the non-transduced MSCs.

19Fc[FUT7⁺] favored MSC adhesion to P-selectin, while FUT7-overexpression enhanced binding to E-selectin. In cell adhesion studies performed under fluid flow, the MSCs bearing 19Fc[FUT7⁺] bound both substrates bearing recombinant P-selectin (Fig. 6D) and CHO-P cells (Fig. 6E). The FUT7 transduced MSCs, on the other hand, bound P-selectin-Fc substrates but not CHO-P cells. Thus 19Fc[FUT7⁺] coupling is a more robust strategy to enhance P-selectin dependent MSC adhesion. The anti-PSGL-1 mAb KPL-1 blocked all cell binding mediated by 19Fc[FUT7⁺] to P-selectin under flow. This mAb, however, did not alter MSC-FUT7 binding to P-selectin-Fc suggesting that FUT7 may fucosylate additional glycoproteins on MSCs that also recognize P-selectin. Binding to both E-selectin-Fc (Fig. 6F) and stimulated HUVECs (Fig. 6G) was low in the 19Fc[FUT7⁺] coupled MSCs but this

interaction was robustly enhanced in the MSC-FUT7. Blocking studies with the anti-E-selectin mAb P2H3 confirm that FUT7 over-expression specifically enhanced stem cell adhesion to E-selectin on the stimulated endothelium.

3.7 Ischemia-reperfusion in swine

To determine if stem cells modified to enhance selectin binding affinity are safe *in vivo* and can be retained distal to the site of coronary infusion, pilot studies were undertaken in three 38kg Yorkshire swine (Fig. 7). CDCs were used for these runs since they more closely resemble endogenous cardiac cells compared to the MSCs. These cells were transduced to overexpress FUT7 using the strategy outlined in Fig. 6, scaled up and then functionalized with PPG and 19Fc[FUT7⁺] just prior to infusion into animals. 80-90% of the cells were stably DsRed positive at the time of injection. Here, a brief 10 min. occlusion of the LAD followed by 30 min. reperfusion was performed to elicit increased selectin expression in the absence of irreversible injury [32, 33]. 8×10^6 functionalized CDCs were then infused proximal to the occlusion site. There was no untoward hemodynamic change or electrocardiographic evidence of myocardial ischemia, and thus the cells and procedure itself was safe and reproducible. Animals were sacrificed and tissue sections were collected 3h after stem cell infusion.

Quantitative real-time PCR of genomic DNA isolated from the LAD, circumflex and right coronary artery regions confirmed the localization of the functionalized stem cells in the LAD region (Fig. 7). Here, 15 copies of the FUT7 gene were observed per 100ng of female recipient DNA in the LAD region (Fig. 7A). FUT7 copy numbers measured in the circumflex and right coronary artery were lower than or equal to both the no template control and negative female tissue sample that lacked the functionalized donor cells. Based on quantitative analysis of the male SRY gene, we estimate a 1.2:1 ratio of the FUT7:SRY genes in the LAD tissue suggesting that the average retained CDC had ~1 copy of the FUT7 gene (Fig. 7B). Further, based on the PCR results, the FUT7 standard/calibration curve and tissue mass of the LAD region it is estimated that 28% of the infused functionalized CDCs were retained in this region at 3h (Fig. 7C). Overall, the large animal pilot study confirms the feasibility of using selectinligand functionalized CDCs *in vivo*.

4. Discussion

Optimizing cellular homing is an important aspect of stem cell therapy since clinical studies [12] and also mouse experiments [13] show a positive correlation between engraftment efficiency and recovery. In the REPAIR-AMI clinical trial, the migratory capacity of bone-marrow progenitor cells delivered to patients following an acute myocardial infarction was an indicator of event-free survival at the 5-year follow up [12]. This result suggests that improved cellular homing benefits patient outcome. While stem cells have been reported to home to injured tissue [34], the exact mechanism is not well understood and tissue uptake could be promoted by passive engraftment through the leaky cardiac microcirculation following injury such as ischemia-reperfusion [35]. Cell surface expression levels of adhesion molecules that promote retention like VLA-4 and the chemokine receptor CXCR4 are often low and variable among stem cells due to the different cell isolation and culturing

protocols. Thus, one cannot rely solely on endogenous molecules for efficient homing to sites of regenerative therapy [36].

To overcome the above limitations, this manuscript presents two complementary methods to functionalize MSCs and CDCs with selectin-ligands for cardiology applications. The first involves the rapid and reversible, non-covalent coupling of a recombinant PSGL-1 variant, 19Fc[FUT7⁺], onto the stem cell surface. In the second, the enzyme FUT7 is over-expressed in these cells. Whereas the first strategy was superior for P-selectin engagement, the latter was the preferred method for binding E-selectin. These observations are consistent with prior literature that show that while sLe^X is abundantly expressed on various leukocyte glycoproteins, the core-2 sLe^X located at the anionic N-terminus of PSGL-1 is a superior P-selectin ligand due to selectin interactions with sulfated tyrosine residues on the PSGL-1 peptide-backbone [26, 37]. Unlike P-selectin, E-selectin is more promiscuous and it binds a number of sialofucosylated ligands [38]. Besides *in vitro* validation, this manuscript also presents *in vivo* data in a large animal model demonstrating the safety and feasibility of this cell delivery modality.

With regard to 19Fc[FUT7⁺], a unique feature of this fusion protein is that it contains a selectin-binding O-glycan at the N-terminus in the context of the natural PSGL-1 protein scaffold [26]. The decoration of stem cells with this molecule allows the direct and specific capture of the cells from flow onto P-selectin bearing substrates. This is unlike previous selectin adhesion approaches that coupled either sialyl Lewis-X (sLe^X) [39, 40], E-selectin binding peptides [41] or performed *ex vivo* fucosylation [27] since these other studies only report the continuous rolling of stem cells after they were initially captured under static conditions (i.e. stop flow), but not the direct capture of cells from shear flow. The latter condition is an important requirement for the systemic infusion method since stop-flow methods can have detrimental effects and do not take advantage of the blood rheology that may promote stem cell margination to the vessel wall [19].

The lipid-based coupling of 19Fc[FUT7⁺] to stem cells is a minimally obtrusive and rapid process. It involves two simple incubation steps that can take as little as 45 min to accomplish. The modification is temporary and maximal immediately after surface functionalization. 19Fc[FUT7⁺] levels decrease thereafter with no expression at 48h. Due to the temporary nature of this modification, this is an ideal strategy for targeted delivery that does not alter either stem cell proliferation or expression of mesenchymal markers (CD90 and CD105). When compared to other methods that use genetic alterations by viral transduction [42, 43], this type of cell coupling also has the advantage of being mild, allowing the cells to exhibit enhanced adhesion properties only during the cell delivery phase. While FUT7 expression in the current work does use lentivirus, non-viral methods can be used with similar outcome in the future.

Both the MSCs and CDCs could be modified with the PSGL-1 protein using either PPG or DPG. In studies that compared the relative efficacy of these lipids to facilitate cell rolling, single-tailed PPG provided a more robust platform for the development of cellular therapeutics compared to the two-tailed DPG. In this regard, single-tailed lipids have been used by us and others to couple molecules onto stem cell surfaces [25, 44]. Function-Spacer-

Lipid (FSL) constructs utilizing a two-tailed lipid, DOPE, have also been used commercially to couple various groups to cell and virion surfaces [45, 46]. Upon performing a systematic comparison of the two approaches, our data show that PPG requires less time and results in more stable lipid incorporation compared to DPG. Cells bearing 19Fc[FUT7⁺] using PPG also displayed more robust cell adhesion to P-selectin. While not all of the available two-tailed lipids with varying lengths or degrees of saturation were examined in this study, the data thus far support the use of PPG for the delivery of cellular therapeutics.

Among the stem-cell types, MSCs had better incorporation of the cell surface modifications compared to CDCs. This resulted in better capture and adhesion of the functionalized MSCs under flow. As the overall size of both cell types is similar, the data suggest that the extent of lipid intercalation may be cell-type specific. In this context, the membrane composition of the two cell types may differ due to their individual sources in the body, isolation protocols, and growth media.

5. Conclusion

In conclusion, the current manuscript presents a systematic set of strategies to functionalize heterologous cell types with ligands for P- and E-selectin. These strategies are based on our current knowledge of selectin adhesion biology, especially the physiological selectin glycoprotein ligands that have been identified to date. The procedure has been scaled up to a large animal model thus confirming that this is a cost-effective strategy for future clinical application. Indeed prior to clinical translation, additional biomaterial modifications will be necessary including the elimination of protein G from the non-covalent immobilization strategy and the use of non-lentiviral approaches to over-express FUT7 in stem cells transiently only at the time of cellular therapeutic delivery. Additionally, while previous studies document the ability of stem cells to transmigrate across the endothelium [27, 47, 48], some modifications may be necessary to enhance this second step as well. Finally, *in vivo* studies are currently underway in our laboratory using clinically relevant large animal models of myocardial infarction to determine if surface modifications described in the current work can enhance cellular uptake and cardiac repair. This line of investigation will determine whether the targeting of glycoengineered cells to the dysfunctional myocardium can enhance the uptake of stem cells following intracoronary injection under continuous flow, improve clinical efficacy in patients with cardiac injury and reduce the total number of cells needed for successful regenerative therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

C.Y.L. was supported by a T32 NIH Ruth L. Kirschstein Postdoctoral Research Training Grant. Additional funding support was provided by NYSTEM contract CO24282 and NIH grants HL55324, HL61610, HL63014, F32HL-114335, and HL103411. Confocal images were acquired at the University at Buffalo North Campus Imaging Facility supported by the NSF-MRI Grant DBI 0923133.

References

1. Karantalis V, DiFede DL, Gerstenblith G, Pham S, Symes J, Zambrano JP, et al. Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: The Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial. *Circ Res*. 2014 Apr 11; 114(8):1302–10. [PubMed: 24565698]
2. Bartunek J, Behfar A, Dolatabadi D, Vanderheyden M, Ostojic M, Dens J, et al. Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. *J Am Coll Cardiol*. 2013 Jun 11; 61(23):2329–38. [PubMed: 23583246]
3. Malliaras K, Makkar RR, Smith RR, Cheng K, Wu E, Bonow RO, et al. Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CARDiosphere-Derived aUTologous stem Cells to reverse ventricUlar dySfunction). *J Am Coll Cardiol*. 2014 Jan 21; 63(2):110–22. [PubMed: 24036024]
4. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*. 2009 Dec 8; 54(24):2277–86. [PubMed: 19958962]
5. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999 Apr 2; 284(5411):143–7. [PubMed: 10102814]
6. Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. 2007 Feb 20; 115(7):896–908. [PubMed: 17283259]
7. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest*. 1999 Mar; 103(5):697–705. [PubMed: 10074487]
8. Gago-Lopez N, Awaji O, Zhang Y, Ko C, Nsair A, Liem D, et al. THY-1 Receptor Expression Differentiates Cardiosphere-Derived Cells with Divergent Cardiogenic Differentiation Potential. *Stem Cell Reports*. 2014 May 6; 2(5):576–91. [PubMed: 24936447]
9. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002 Jan 1; 105(1):93–8. [PubMed: 11772882]
10. Weil BR, Canty JM Jr. Stem cell stimulation of endogenous myocyte regeneration. *Clin Sci (Lond)*. 2013 Aug; 125(3):109–19. [PubMed: 23577634]
11. Xie Y, Ibrahim A, Cheng K, Wu Z, Liang W, Malliaras K, et al. Importance of cell-cell contact in the therapeutic benefits of cardiosphere-derived cells. *Stem Cells*. 2014 Sep; 32(9):2397–406. [PubMed: 24802280]
12. Assmus B, Leistner DM, Schachinger V, Erbs S, Elsasser A, Haberbosch W, et al. Long-term clinical outcome after intracoronary application of bone marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival. *Eur Heart J*. 2014 May 14; 35(19):1275–83. [PubMed: 24569031]
13. Liu J, Narsinh KH, Lan F, Wang L, Nguyen PK, Hu S, et al. Early stem cell engraftment predicts late cardiac functional recovery: preclinical insights from molecular imaging. *Circ Cardiovasc Imaging*. 2012 Jul; 5(4):481–90. [PubMed: 22565608]
14. Campbell NG, Suzuki K. Cell delivery routes for stem cell therapy to the heart: current and future approaches. *J Cardiovasc Transl Res*. 2012 Oct; 5(5):713–26. [PubMed: 22648235]
15. du Pre BC, Doevendans PA, van Laake LW. Stem cells for cardiac repair: an introduction. *J Geriatr Cardiol*. 2013 Jun; 10(2):186–97. [PubMed: 23888179]
16. Johnston PV, Sasano T, Mills K, Evers R, Lee ST, Smith RR, et al. Engraftment, differentiation, and functional benefits of autologous cardiosphere-derived cells in porcine ischemic cardiomyopathy. *Circulation*. 2009 Sep 22; 120(12):1075–83. [PubMed: 19738142]

17. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet*. 2012 Mar 10; 379(9819):895–904. [PubMed: 22336189]
18. Tossios P, Krausgrill B, Schmidt M, Fischer T, Halbach M, Fries JW, et al. Role of balloon occlusion for mononuclear bone marrow cell deposition after intracoronary injection in pigs with reperfused myocardial infarction. *Eur Heart J*. 2008 Aug; 29(15):1911–21. [PubMed: 18544545]
19. Fedosov DA, Gompper G. White blood cell margination in microcirculation. *Soft Matter*. 2014 May 7; 10(17):2961–70. [PubMed: 24695813]
20. Suzuki G, Iyer V, Lee TC, Canty JM Jr. Autologous mesenchymal stem cells mobilize cKit+ and CD133+ bone marrow progenitor cells and improve regional function in hibernating myocardium. *Circ Res*. 2011 Oct 14; 109(9):1044–54. [PubMed: 21885831]
21. Suzuki G, Weil BR, Leiker MM, Ribbeck AE, Young RF, Cimato TR, et al. Global intracoronary infusion of allogeneic cardiosphere-derived cells improves ventricular function and stimulates endogenous myocyte regeneration throughout the heart in swine with hibernating myocardium. *PLoS One*. 2014; 9(11):e113009. [PubMed: 25402428]
22. Kriegstein CF, Granger DN. Adhesion molecules and their role in vascular disease. *Am J Hypertens*. 2001 Jun; 14(6 Pt 2):44S–54S. [PubMed: 11411765]
23. Jones SP, Trocha SD, Strange MB, Granger DN, Kevil CG, Bullard DC, et al. Leukocyte and endothelial cell adhesion molecules in a chronic murine model of myocardial reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2000 Nov; 279(5):H2196–201. [PubMed: 11045953]
24. Chukwuemeka AO, Brown KA, Venn GE, Chambers DJ. Changes in P-selectin expression on cardiac microvessels in blood-perfused rat hearts subjected to ischemia-reperfusion. *Ann Thorac Surg*. 2005 Jan; 79(1):204–11. [PubMed: 15620944]
25. Lo CY, Antonopoulos A, Dell A, Haslam SM, Lee T, Neelamegham S. The use of surface immobilization of P-selectin glycoprotein ligand-1 on mesenchymal stem cells to facilitate selectin mediated cell tethering and rolling. *Biomaterials*. 2013 Nov; 34(33):8213–22. [PubMed: 23891082]
26. Lo CY, Antonopoulos A, Gupta R, Qu J, Dell A, Haslam SM, et al. Competition between core-2 GlcNAc-transferase and ST6GalNAc-transferase regulates the synthesis of the leukocyte selectin ligand on human P-selectin glycoprotein ligand-1. *J Biol Chem*. 2013 May 17; 288(20):13974–87. [PubMed: 23548905]
27. 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 Feb; 14(2):181–7. [PubMed: 18193058]
28. Xia L, McDaniel JM, Yago T, Doeden A, McEver RP. Surface fucosylation of human cord blood cells augments binding to P-selectin and E-selectin and enhances engraftment in bone marrow. *Blood*. 2004 Nov 15; 104(10):3091–6. [PubMed: 15280192]
29. Buffone A Jr, Mondal N, Gupta R, McHugh KP, Lau JT, Neelamegham S. Silencing alpha1,3-fucosyltransferases in human leukocytes reveals a role for FUT9 enzyme during E-selectin-mediated cell adhesion. *J Biol Chem*. 2013 Jan 18; 288(3):1620–33. [PubMed: 23192350]
30. Mondal N, Buffone A Jr, Stolfi G, Antonopoulos A, Lau JT, Haslam SM, et al. ST3Gal-4 is the primary sialyltransferase regulating the synthesis of E-, P-, and L-selectin ligands on human myeloid leukocytes. *Blood*. 2015 Jan 22; 125(4):687–96. [PubMed: 25498912]
31. Hong KU, Li QH, Guo Y, Patton NS, Mokhtar A, Bhatnagar A, et al. A highly sensitive and accurate method to quantify absolute numbers of c-kit+ cardiac stem cells following transplantation in mice. *Basic Res Cardiol*. 2013 May; 108(3):346. [PubMed: 23549981]
32. Kloner RA, Jennings RB. Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 1. *Circulation*. 2001 Dec 11; 104(24):2981–9. [PubMed: 11739316]
33. Palazzo AJ, Jones SP, Anderson DC, Granger DN, Lefer DJ. Coronary endothelial P-selectin in pathogenesis of myocardial ischemia-reperfusion injury. *Am J Physiol*. 1998 Nov; 275(5 Pt 2):H1865–72. [PubMed: 9815095]
34. Rustad KC, Gurtner GC. Mesenchymal Stem Cells Home to Sites of Injury and Inflammation. *Adv Wound Care (New Rochelle)*. 2012 Aug; 1(4):147–52. [PubMed: 24527296]

35. Kean TJ, Lin P, Caplan AI, Dennis JE. MSCs: Delivery Routes and Engraftment, Cell-Targeting Strategies, and Immune Modulation. *Stem Cells Int.* 2013; 2013:732742. [PubMed: 24000286]
36. Park JS, Suryaprakash S, Lao YH, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. *Methods.* 2015 Mar 11.
37. Sako D, Comess KM, Barone KM, Camphausen RT, Cumming DA, Shaw GD. A sulfated peptide segment at the amino terminus of PSGL-1 is critical for P-selectin binding. *Cell.* 1995 Oct 20; 83(2):323–31. [PubMed: 7585949]
38. Mondal N, Buffone A Jr, Neelamegham S. Distinct glycosyltransferases synthesize E-selectin ligands in human vs. mouse leukocytes. *Cell Adh Migr.* 2013 May-Jun;7(3):288–92. [PubMed: 23590904]
39. Sarkar D, Vemula PK, Zhao W, Gupta A, Karnik R, Karp JM. Engineered mesenchymal stem cells with self-assembled vesicles for systemic cell targeting. *Biomaterials.* 2010 Jul; 31(19):5266–74. [PubMed: 20381141]
40. Sarkar D, Spencer JA, Phillips JA, Zhao W, Schafer S, Spelke DP, et al. Engineered cell homing. *Blood.* 2011 Dec 15; 118(25):e184–91. [PubMed: 22034631]
41. Cheng H, Byrsk-Bishop M, Zhang CT, Kastrup CJ, Hwang NS, Tai AK, et al. Stem cell membrane engineering for cell rolling using peptide conjugation and tuning of cell-selectin interaction kinetics. *Biomaterials.* 2012 Jul; 33(20):5004–12. [PubMed: 22494889]
42. 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 infarcted myocardium improves cardiac performance. *Mol Ther.* 2008 Mar; 16(3):571–9. [PubMed: 18253156]
43. Cho YH, Cha MJ, Song BW, Kim IK, Song H, Chang W, et al. Enhancement of MSC adhesion and therapeutic efficiency in ischemic heart using lentivirus delivery with periostin. *Biomaterials.* 2012 Feb; 33(5):1376–85. [PubMed: 22112759]
44. Kim SA, Peacock JS. The use of palmitate-conjugated protein A for coating cells with artificial receptors which facilitate intercellular interactions. *J Immunol Methods.* 1993 Jan 14; 158(1):57–65. [PubMed: 8429217]
45. Blake DA, Bovin NV, Bess D, Henry SM. FSL constructs: a simple method for modifying cell/virion surfaces with a range of biological markers without affecting their viability. *J Vis Exp.* 2011; (54)
46. Korchagina E, Tuzikov A, Formanovsky A, Popova I, Henry S, Bovin N. Toward creating cell membrane glyco-landscapes with glycan lipid constructs. *Carbohydr Res.* 2012 Jul 15; 356:238–46. [PubMed: 22551471]
47. Teo GS, Ankrum JA, Martinelli R, Boetto SE, Simms K, Sciuto TE, et al. Mesenchymal stem cells transmigrate between and directly through tumor necrosis factor- α -activated endothelial cells via both leukocyte-like and novel mechanisms. *Stem Cells.* 2012 Nov; 30(11):2472–86. [PubMed: 22887987]
48. Ko IK, Kim BG, Awadallah A, Mikulan J, Lin P, Letterio JJ, et al. Targeting improves MSC treatment of inflammatory bowel disease. *Molecular Therapy.* 2010 Jul; 18(7):1365–72. [PubMed: 20389289]

Fig. 1. Coupling of 19Fc/19Fc[FUT7⁺] onto MSCs and CDCs

A Chemical structure of palmitic acid (P) and dodecanyl DOPE (D). **B.** MSCs/CDCs (with or without lentiviral FUT7 over-expression) were incubated with either palmitate protein G (PPG) or dodecanyl DOPE protein G (DPG) to obtain “MSC/CDC + PPG” and “MSC/CDC + DPG”, respectively. After washing, these cells were incubated with 19Fc or 19Fc[FUT7⁺] to obtain “MSC/CDC + PPG/DPG + 19Fc/19Fc[FUT7⁺]”. **C.** The binding of FITC conjugated goat anti-human (GαH) IgG F(ab')₂ revealed the presence of 19Fc on the surface of CDCs and MSCs. This Ab primarily binds the Fc section of 19Fc. Although the F(ab')₂ antibody lacks the Fc domain, some protein G binding to the Ab was detected.

Fig. 2. Optimizing lipid-protein G incubation conditions

All panels used 100 μg/ml 19Fc after lipid incubation and FITC conjugated GaH IgG F(ab')₂ as the detection antibody. **A.** and **B.** Temperature was varied during the lipid incubation step for the CDCs (**A**) and MSCs (**B**), $n = 3-5$. Cells were treated with 100 μg/ml PPG/DPG at the indicated temperature followed by incubation with 19Fc. 19Fc incorporation was most effective at 37°C. **C.** and **D.** The lipid incubation time was varied for CDCs (**C**) and MSCs (**D**), $n = 5-7$. Cells were treated with 100 μg/ml PPG/DPG at the indicated time followed by incubation with 19Fc. For CDCs, PPG showed higher incorporation compared to DPG at the earlier time points. **E.** and **F.** The concentration of lipid-protein G complex was varied in CDCs (**E**) and MSCs (**F**), $n = 3$. Cells were treated with PPG/DPG at the indicated concentration for 1h followed by incubation with 19Fc. 19Fc incorporation increased approximately linearly with PPG/DPG concentration. In all panels, $* P < 0.05$ for the indicated comparisons.

Fig. 3. Topography and time course of 19Fc binding

19Fc bound onto CDCs (**A** and **C**) and MSCs (**B** and **D**) as detected by FITC conjugated GαH IgG F(ab')₂ antibody. **A.** and **B.** Confocal micrographs show the cell surface localization of 19Fc immediately after treatment of the cells with 100 μg/ml PPG/DPG for 1h at 37°C, followed by 100 μg/ml 19Fc. Scale bar = 20 μm. **C.** and **D.** Dissociation of surface coupled 19Fc was monitored for 4 days after preparation, n = 3. 19Fc binding to cells was reduced to baseline at 48h. In all panels, * *P* < 0.05 compared to “CDC/MS + DPG + 19Fc”.

Fig. 4. Effect of coupling on cell surface stem cell markers and proliferation

Treatment of either CDCs or MSCs with PPG or DPG and 19Fc under ‘standard conditions’ did not adversely affect cell surface expression of CD105, CD90 or cell proliferation (assessed using BrdU) measured at **A.** Day 1 after treatment for CDCs (top) and MSCs (bottom), and **B.** Day 7 after treatment for CDCs (top) and MSCs (bottom), n=3.

Fig. 5. Cell binding to P-selectin in a microfluidic flow chamber under continuous flow
 Interacting cell density was quantified for CDCs (**A** and **B**) and MSCs (**C** and **D**), $n = 3-11$. 19Fc[FUT7⁺] or 19Fc were immobilized using PPG/DPG to CDCs/MSCs under 'standard conditions'. **A** and **C**. 19Fc[FUT7⁺] could effectively bind P-selectin under all fluid shear stresses (2-6 dyne/cm²) when coupled using PPG. Immobilization using DPG resulted in less adhesion to P-selectin at all shear stresses. **B** and **D**. Cell adhesion was blocked by anti-PSGL-1 mAb KPL1 and anti-P-selectin mAb G1, thus confirming the specificity of the measured interaction (2 dyne/cm²). * $P < 0.05$ with respect to all other treatments except where indicated by n.s. (not significant).

Fig. 6. Over-expression of FUT7 in MSCs

A. pCSCG lentiviral vector used to over-express FUT7-DsRed fusion protein in MSCs and CDCs. **B.** Fluorescence microscopy image of untransduced MSCs ('MSC') and FUT7-transduced MSCs ('MSC-FUT7'). Only the MSC-FUT7 cells display red fluorescence. **C.** Flow cytometry analysis of DsRed fluorescence, AAL-FITC and CSLEX-1 binding to MSC-FUT7 cells compared to untransduced MSCs. Negative controls are presented using dotted histograms: isotope controls for mAb CSLEX-1 panel and competitive inhibitor 100mM fucose in the case of AAL-FITC. Mean $\pm$ SEM data are provided next to representative cytometry histograms. **D-G.** MSC, MSC-FUT7 or cells with surface coupled 19Fc[FUT7⁺] via PPG were perfused over substrates composed of either recombinant P-selectin-Fc (**D**), CHO-P cells (**E**), recombinant E-selectin-Fc (**F**) or 4h IL-1 β stimulated HUVEC monolayers (**G**) at a wall shear stress of 1 dyn/cm². Blocking mAbs used to confirm binding specificity include anti-PSGL-1 mAb KPL-1 which blocks 19Fc[FUT7⁺],

anti-P-selectin mAb G1 and anti-E-selectin mAb P2H3. $*P<0.05$ with respect to all other conditions except that treatments indicated by * are not different from each other. One exception is indicated in panel F.

Fig. 7. Swine experiments

CDCs over-expressing FUT7 and coupled with ¹⁹Fc[FUT7⁺] were infused in the LAD following brief ischemia-reperfusion. **A.** Real-time PCR quantified the retention of the infused CDCs in LAD, circumflex and right coronary artery supplied myocardium by measuring FUT7 gene copy numbers in excised tissue. FUT7 copy numbers in circumflex and right coronary artery are close to negative control suggesting that the infused cells are localized in the LAD region; **B.** Copies of FUT7/Y-chromosome in LAD region; **C.** Total donor cells retained/g tissue in LAD infused region. **P*<0.05 with respect to all other regions.