Biomaterials 74 (2016) 19–30

Contents lists available at ScienceDirect

Biomaterials

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

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

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ARTICLE INFO

Article history:
Received 14 September 2015
Accepted 15 September 2015
Available online 25 September 2015

Keywords:
Mesenchymal stem cells
Adhesion molecule
Blood flow
Cell adhesion
Recombinant protein
Selectin
PSGL-1
Sialyl Lewis-X

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 α(1,3) fucosyltransferse 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, α(1,3)fucosylation was necessary for robust binding to E-selectin and inflamed endothelial cells under shear. Pilot studies confirm 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.

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

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http://dx.doi.org/10.1016/j.biomaterials.2015.09.026
0142-9612/

approach results in substantial stem cell retention in the heart tissue with >90% of the injected cells no longer present 24 h 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 24 h 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 express the α(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 glycoproptein 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 4 h 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 ± 4.7 μm and 18.2 ± 5.1 μm (mean ± 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 extract 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 × 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 α(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