Selection of chemotactic adipose-derived stem cells using a microfluidic gradient generator

Supplementary Information

Kanmani Natarajan[a,b], Chantal Tian[a], Bo Xiang[c,d], Chao Chi,[d,e,f] Jixian Deng[d,f], Rundi Zhang[a], Darren H. Freed[g], Rakesh C. Arora[h], Ganghong Tian[d,f,*], Francis Lin[a,b,i,j,*]

[a]Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

[b]Department of Biological Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

[c]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, R3E 0T6, Canada

[d]National Research Council, Winnipeg, MB, R3B 1Y6, Canada

[e]Department of Cardiac Surgery, the First Affiliated Hospital, Harbin Medical University, Harbin, China

[f]Department of Physiology and Pathophysiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB, R3E 0J9, Canada

[g]Division of Cardiac Surgery, University of Alberta Hospital

[h]Cardiac Science Program, St. Boniface General Hospital, Winnipeg, Manitoba, Canada

[i]Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

[j]Department of Immunology, University of Manitoba, Winnipeg, MB, R3E 0T5, Canada

[*] To whom the correspondence should be addressed

flin@physics.umanitoba.ca for F. Lin

Ganghong.Tian@nrc-cnrc.gc.ca for G.H. Tian

S.1. Immunofluorescence staining and imaging of EGFR in HC and LC

HC and LC ASCs were grown on sterile glass coverslips overnight at 37°C. The cells were washed briefly with PBS and fixed with 4% paraformaldehyde for 10-20 minutes at room temperature. The fixed samples were washed with PBS followed by rinsing in 0.05% Tween20. They were then blocked with 5% BSA in PBS-Tween20 for 30 min at room temperature to block non-specific binding of immunoglobulin The blocked samples were stained with the primary antibody (EGFR (1005):sc-03, Santa Cruz Biotechnology) and left overnight at 4°C. They were rinsed with PBS-Tween20 and stained with the secondary antibody (Alexa Fluor 488 Goat Anti-Rabit IgG (H+L) antibody, Life Technologies) for 30 min at room temperature. The samples were rinsed with PBS-Tween20 and counterstained with DAPI for 15 min in dark at room temperature. Fluorescent images were taken using a Zeiss microscope. The staining shows qualitative difference in EGFR expression between the HC and LC ASCs by visualization (Fig. S1).

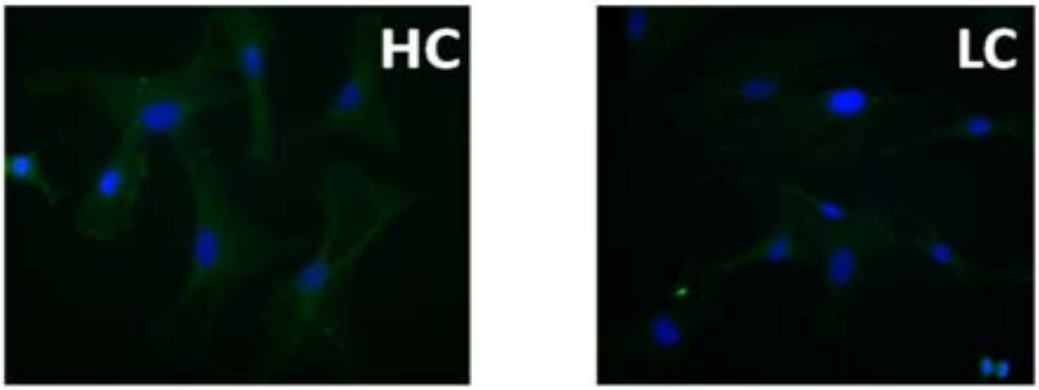


Fig. S1. Immunofluorescence staining images of surface expression of EGFR in HC and LC.

S.2. Characterizations of EGF-induced Erk1/2 phosphorylation in HC and LC

Detailed method of phospho FACS for measuring Erk1/2 phosphorylation of HC and LC ASCs was described in the Materials and Methods section in the main manuscript. The key result was reported in Fig. 5D in the main manuscript. Here we describe the data in more details (Fig. S2). Without EGF stimulation, HC and LC show similar level of Erk1/2 phosphorylation. EGF stimulation increased the level of Erk1/2 phosphorylation in both HC and LC. Finally, EGF stimulation induced higher level of Erk1/2 phosphorylation in HC than LC.

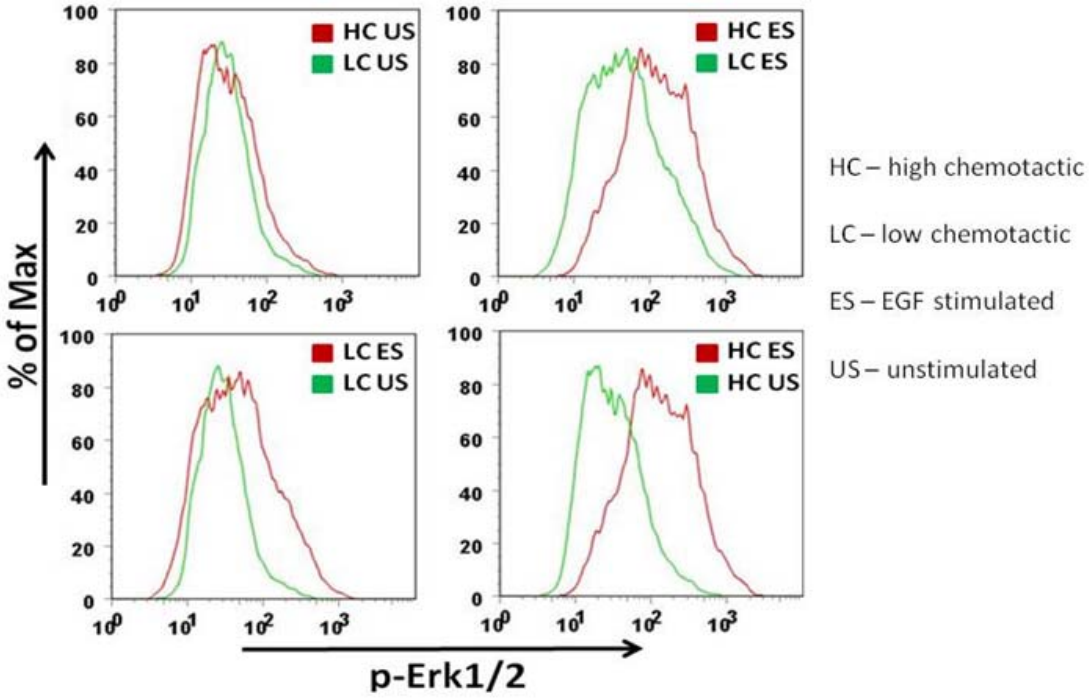
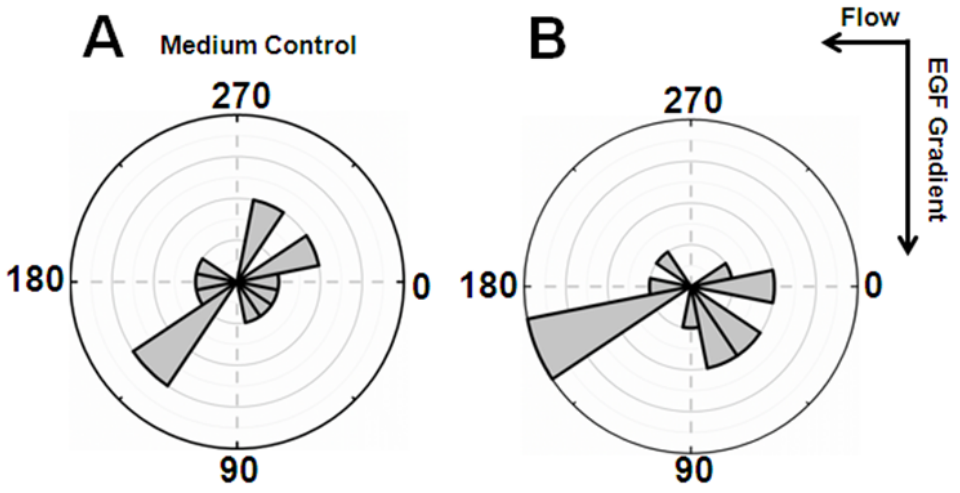


Fig. S2. Erk1/2 activation in HC and LC with or without EGF stimulation measured by phospho FACS.

S.3. Migration of human adipose derived stem cells in an EGF gradient in microfluidic devices

We analyzed the migration of human ASCs in response to EGF. The human ASCs were excised from the mediastinal fat deposit of the human patients during cardiac surgery in St. Boniface General Hospital in Winnipeg. The average age of the patients is 61 ±10. The same method of patterning and chemotaxis as described in the Materials and Methods section in the main manuscript was followed. Time-lapse images were taken every 2 minutes for 7.5 hrs. The human ASCs also exhibited similar migration to EGF as rat ASCs (Fig. S3). When exposed to an EGF gradient, the human ASCs showed significantly higher C.I. compared to the medium control whereas the migratory speed is comparable. Three independent experiments were repeated for each condition and similar results were obtained. Thus, EGF gradient induced chemotaxis of hASCs



C

	% of cells moving downward	Chemotactic Index	Speed (μm/min)
EGF	79	0.14 ± 0.02	0.89 ± 0.06
Control	50	0.02 ± 0.04	1.00 ± 0.12

Fig. S3. Quantitative analysis of human ASCs migration in medium control or an EGF gradient in the microfluidic devices. (A-B) Angular histograms show hASCs migrate randomly in medium control and towards EGF in the presence of gradient. **(C)** Percentage of cells moving toward the gradient, Chemotactic Index (C.I.) and migration speed of hASCs in medium control or EGF gradient.

S.4. Preliminary results of rat ASCs migration in a gradient of myocardial extraction from injured rat hearts using microfluidic devices

Instead of using EGF as the chemoattractant, we further tested rat ASCs migration in response to a gradient of myocardial extraction from globally injured rat hearts using microfluidic devices. To prepare the myocardial extraction, Sprague-Dawley rats were euthanized with overdose of isoflurane. The resulting cardiac arrest was maintained for 2 hrs to ensure global and irreversible myocardial injury. The injured rat hearts were then removed from animals and rinsed with PBS three times at 4°C. Left ventricular myocardium was minced into small pieces in 4°C Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, Inc., Burlington, Canada). After homogenization with a handheld glass-glass Dounce tissue grinder, the suspension was centrifuged at 12000g for 15 minutes. The supernatant was subsequently concentrated with Amicon Ultra-15 Centrifugal Filter Units (Millipore Corporation, Billerica, MA). The concentrated extraction was stored at –80°C till usage. Immediately before chemotaxis experiments, the concentrated extraction was diluted ten times with complete medium. Chemotaxis experiments (2 independent experiments) were performed for 23 hrs using the microfluidic method and the cell migration data were analyzed. Our current results show that more cells migrated toward the myocardium extraction gradient (Fig. S4), suggesting the potential of the microfluidic methods for studying ASCs migration to target tissues and selecting target-directed ASCs.

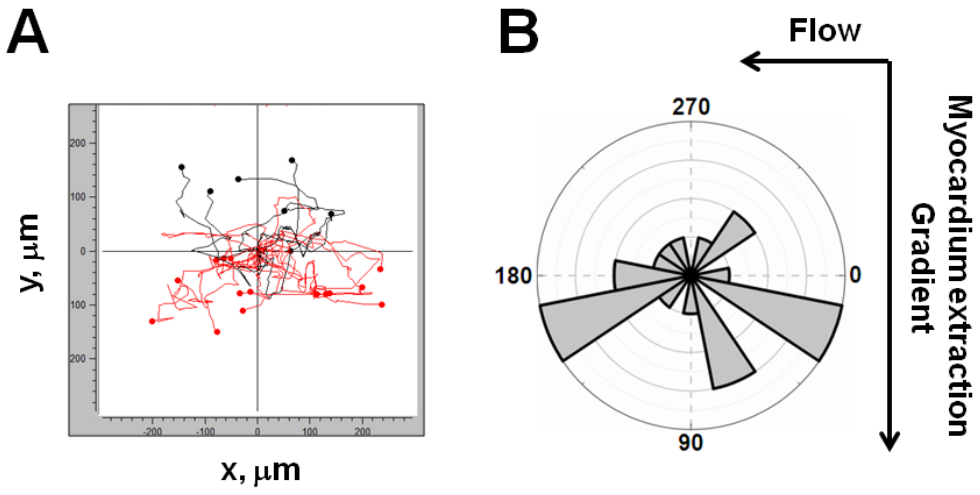


Fig. S4. ASCs migration in a gradient of myocardial extraction from injured rat hearts using microfluidic devices. (A) Cell tracks with the starting positions normalized to a common origin. Red tracks are cells migrated towards the gradient; black tracks are cells migrated away from the gradient. **(B)** Angular histogram of the cells in **(A)**.