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Fucosylated umbilical cord blood hematopoietic stem cell expansion on selectin-coated scaffolds

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Abstract

Despite the advantages of transplantation of umbilical cord blood's (UCB's) hematopoietic stem cells (uHSCs) for hematologic malignancy treatment, there are two major challenges in using them: (a) Insufficient amount of uHSCs in a UCB unit; (b) a defect in uHSCs homing to bone marrow (BM) due to loose binding of their surface glycan ligands to BM's endothelium selectin receptors. To overcome these limitations, after poly L-lactic acid (PLLA) scaffold establishment and incubation of uHSCs with fucosyltransferase-VI and GDP-fucose, ex vivo expansion of these cells on selectin-coated scaffold was done. The characteristics of the cultured fucosylated and nonfucosylated cells on a two-dimensional culture system, PLLA, and a selectin-coated scaffold were evaluated by flow cytometry, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, colony-forming unit (CFU) assay, and CXCR4 expression at the messenger RNA and protein levels. According to the findings of this study, optimized attachment to the scaffold in scanning electron microscopy micrograph, maximum count of CFU, and the highest 570 nm absorption were observed in fucosylated cells expanded on selectin-coated scaffolds. Furthermore, real-time polymerase chain reaction showed the highest expression of the CXCR4 gene, and immunocytochemistry data confirmed that the CXCR4 protein was functional in this group compared with the other groups. Considered together, the results showed that selectin-coated scaffold could be a supportive structure for fucosylated uHSC expansion and homing by nanotopography. Fucosylated cells placed on the selectin-coated scaffold serve as a basal surface for cell–cell interaction and more homing potential of uHSCs. Accordingly, this procedure can also be considered as a promising technique for the hematological disorder treatment and tissue engineering applications.

KEYWORDS

3D culture, cord blood stem cells, fucosyltransferase, GDP-fucose, poly L-lactic acid

1 | INTRODUCTION

Due to the umbilical cord blood's (UCB's) hematopoietic stem cells' (uHSCs') simple collection, easy access, low rate, and intensity of graft versus host disease, HSC transplantation is used to manage numerous hematological diseases. Accordingly, uHSCs are potent alternative sources for bone marrow (BM) and peripheral blood's HSCs (Bojanic & Golubic Cepulic, 2006). Furthermore, effective transplantation of these cells provides an efficient hematopoiesis process for unhealthy patients without human leukocyte-antigen-matched donors (Aggarwal, Lu, Pompili, & Das, 2012; Bertolini et al., 1998; Kelly, Sola, de Lima, & Shpall, 2009). Despite these advantages, there are some limitations in uHSC transplantation, such as the low content of uHSC in collected UCB, which results in negative effects on their transplantation in adults and children (Ehring et al., 2003; Sotnezova, Andreeva, Grigoriev, & Buravkova, 2016). Another disadvantage of uHSC transplantation is a delay in uHSCs' attachment and homing into their natural niche in comparison with the traditional stem cell sources. According to some previous studies, it has been revealed that in UCB, the ratio of HSCs containing fucose in their surface carbohydrate chain is lower than other sources. So, uHSCs show defects in their homing because of an incomplete carbohydrate chain. HSC surface molecules are essential components for the homing process with a high potential for binding to BM endothelium, which might be managed through a cascade of adhesion molecules achieved by selectin and other receptors expressed on the microvasculature of the BM (Pineault & Abu-Khader, 2015; Timeus et al., 1998; Tung, Parmar, Robinson, De Lima, & Shpall, 2010).

In this regard, P-selectin is a transmembrane glycoprotein expressed by endothelial cells and platelets that mediate tumbling of leukocytes on the endothelial surface and interact with P-selectin glycoprotein ligand (PSGL) on the surface of HSCs (Greenberg, Kerr, & Hammer, 2000; Katayama et al., 2003; Xia et al., 2003). In the fucosylation process, the fucosyl section conveys from guanosine diphosphate (GDP)-fucose to the center of oligosaccharide structures by α1,3-fucosyltransferases (FTs) that catalyze the final step of glycosyl transfer and create a sialyl Lewis X (sLe^X) base. So, to form a strong binding, PSGL must certainly be α1,3-fucosylated (Prenc et al., 2016; Robinson et al., 2012, 2014; Wan et al., 2013).

To overcome these limitations, fucosylation and ex vivo expansion of uHSCs could be effective. Previously, uHSCs were cultured in two-dimensional (2D) culture systems in a single layer of cells with a low surface-to-volume ratio. Therefore, they were not suitable models for studying the cell's surface interactions. So, production of a natural niche based on a nanoscaffold that mimicks the natural niche of uHSC could be a practical approach in this regard (Atashi, Islami, Mortazavi, & Soleimani, 2018). In stem cell biology, the use of 3D culture system for various cell types is more efficient than the 2D culture system. Applying polymeric biomaterials as scaffolds for cell expansion by mimicking the 3D structure of the natural niche improves the efficiency of uHSC transplantation (Pineault & Abu-Khader, 2015).

In this regard, nanofibrous scaffolds have a topographic structure with a high surface-to-volume ratio and mimic the supportive structures of a natural extracellular matrix microenvironment (Mobarra, Soleimani, Pakzad, Enderami, & Pasalar, 2018). For instance, the poly L-lactic acid (PLLA) polymer is wildly used because of its biocompatible and biodegradable potential (Eskandari et al., 2015; Mehta, Kumar, Bhunia, & Upadhyay 2005; Mehta et al., 2015; Pineault & Abu-Khader, 2015). This nanoscaffold could be adapted to individuals quickly without excitation of the immune response (Enderami et al., 2018).

Furthermore, considering the lack of biorecognition sites for uHSCs and poor adhesive qualities, it additionally generates an improper environment for sustaining cells (Carletti, Motta, & Migliaresi, 2011; Celebi, Mantovani, & Pineault, 2011; Mehta et al., 2005). These problems could possibly be resolved by coating the scaffolds with selectin, creating receptors for the fucosylated cell's glycan ligand adhering and survival of HSCs (Colombo, Calcaterra, Cappelletti, Mavilio, & Della Bella, 2013; Dong & Lv, 2016; Greenberg et al., 2000; Hidalgo, Weiss, & Frenette, 2002).

Because the BM is a natural niche of HSCs, appropriate expression of CXCR4 on the surface of HSCs led to correct homing. Regarding that there is a relationship between the more expression of CXCR4 receptor and more homing (Atashi et al., 2018; Kollet et al., 2001; Nie, Han, & Zou, 2008; Wysoczynski et al., 2005), overexpression of CXCR4 is considered as effective homing (Pineault & Abu-Khader, 2015).

In this study, a 2D culture system, a PLLA, and a selectin-coated PLLA were used as scaffolds for fucosylated and nonfucosylated UCB-CD133+ cell (uHSCs) expansion. Then, the homing potential of the cultured cells on scaffolds and 2D culture system was evaluated. Overall, in this study, we proposed to assess the synergic effects of uHSC's fucosylation and selectin-coated PLLA scaffold on uHSCs expansion and homing potential.

2 | MATERIALS AND METHODS

2.1 | CD133+ cells

After obtaining informed consents from normal full-term pregnant women from the Iranian Blood Transfusion Organization, their UCB–HSCs were collected and then diluted with hydroxyethyl starch (Chemie GmbH; Sigma-Aldrich) in a 1:6 ratio to remove red blood cells . The diluted UCB was made to undergo Ficoll-HyPaque (density 1.077 g/ml; Pharmacia-Amersham) density gradient centrifugation to isolate mononuclear cells (MNCs) from the buffy coat. Then, the cells were washed two times with phosphate buffer saline (PBS)/ethylenediaminetetraacetic acid. The UCB-CD133+ cells were then seprated from MNCs via magnetic-assisted cell sorting technology using MACS CD133 Microbead kit (MiltenyiBiotec) based on the manufacturer's guidelines. Finally, the flow cytometry method was used to verify the purity of the isolated cells.

2.2 | Preparation of 3D nanoscaffold and surface modification

For PLLA scaffolds construction via the electrospinning method, after dissolving PLLA (Sigma) in chloroform (9% wt/wt),

N,*N*-dimethylformamide (Sigma) solution was added directly to it (10:1). Then, the PLLA scaffold's surface was improved with plasma treatment via a low frequency plasma generator of 40 kHz frequency, with a cylindrical quartz reactor (Diener Electronics). PLLA scaffolds were sterilized in 70% ethanol, and then placed in 24-well polystyrene plates. Subsequently, the scaffolds were evaluated as two groups:

1) Selectin-coated scaffold: Scaffold coating by selectin through the incubation of the scaffold with 4 μg/ml of P-selectin in the sterilized distilled water (DW) for an hour at room temperature (RT).
2) PLLA scaffold: Finally, the scaffolds were washed with DW.

2.3 | UCB-CD133+ cells treatment by FT-VI

The sLe^X residues are features of fucosylation, which can be detected through flow cytometry by the HECA-452 antibody (BD Biosciences) against cutaneous lymphocyte antigen (CLA). This occurrs via binding the carbohydrate domain of CLA and the sLe^X antigen. The anti-CLA (HECA-452) antibodies (BD Biosciences) or isotype controls were used for cells staining. The FT-VI reaction was performed in the presence of 10^6 CB MNCs, 1 mM of β GDP-Fucose (EMD Biosciences), 1 mM of $MnCl_2$ in 1 ml of Hanks-buffered saline solution including 1% human serum albumin and 20 mU/ml of α1–3 FT-VI (EMD Biosciences). The cells were kept at 37°C for 30 min in an incubator containing 5% CO_2, then washed, and finally counted. CD133+ cells were studied in six treatment groups: fucosylated cells were seeded on (a) 2D culture system, (b) PLLA scaffold, and(c) selectin-coated scaffold; and nonfucosylated cells were seeded on (d) 2D culture system, (e) PLLA scaffold, and (f) selectin-coated scaffold.

2.4 | Confirmation of the immunophenotyping

Evaluation of the UCB-CD133+ cells' purity was done by flow cytometry analysis. At first, Fc receptors of UCB-CD133+ cells were blocked with human immunoglobulin G (IgG; BD Biosciences). Then, 1×10^5 cells were exposed to 10 μl of phycoerythrin (PE)-conjugated CD133 monoclonal antibody (mAb) (MiltenyiBiotec). To determine the sLe^X marker, UCB MNCs were incubated with PE–conjugated anti-human CD133 mAb or control murine IgG (IgG1; BD Biosciences) and with rat anti-sLe^X mAb HECA-452 (IgM; hybridoma from American Type Culture Collection), or rat control IgM (BD Biosciences). HECA-452 bound was detected with fluorescein isothiocyanate (FITC)-labeled goat anti-rat IgM (EMD Biosciences), and HECA-452 bound with FITC-labeled goat anti-rat IgM (EMD Biosciences). All flow cytometry analyses were carried out on a FACSCalibur (BD Biosciences). PE- and FITC-labeled anti-mouse isotype IgG stains were used as compensation controls. The collected data were analyzed using the Cell Quest program (BD Biosciences). Flow cytometry analyses were performed in fucosylated and nonfucosylated cells after 7 days.

2.5 | Modification and characterization of the prepared scaffolds

The hydrophilicity and grafting potential of selectin onto the PLLA scaffold surface was determined using the attenuated total reflection (ATR)–Fourier-transformation infrared technique (FTIR; BOMEM-SRG1100G) spectroscopy. The ATR–FTIR spectrum of selectin-coated PLLA scaffold was compared with the PLLA scaffold.

2.6 | Expansion of the CD133+ cells

A total of 5×10^4 fucosylated and nonfucosylated UCB-CD133+ cells were seeded on a 2D culture system and a nanofiber scaffold in the presence of serum-free medium, Stemspan (Stem Cell Technologies), which included 100 U/ml of penicillin, 100 μg/ml of streptomycin (Life Technologies), 100 ng/ml of stem cell factor, 100 ng/ml of thrombopoietin, 100 ng/ml of Fms-related tyrosine kinase 3 ligand, and antibiotic/antimycotic (penicillin G sodium 100 ug/ml, streptomycin 100 ug/ml). All three growth factors were from Merck Millipore. The cells were seeded in triplicate onto each modified scaffold (mentioned above) put in the middle wells of 24-well tissue culture plates and the 2D culture system and then incubated for 7 days at 37°C and 5% CO_2. Half of the medium was renewed every 48 hr (Denning-Kendall, Singha, Bradley, & Hows, 2003).

2.7 | Viability assay

The biocompatibility of the PLLA scaffold was measured by the 3-(4,5-dimethyl-thiazolyl-2)-2, 5-diphenoltetrazolium bromide (MTT) assay of the fucosylated and nonfucosylated CD133+ cells for 7 days. For this, 3×10^3 cells were seeded on sterilized scaffolds and incubated at 37°C, 5% CO2. After adding 5 mg/ml MTT (Sigma) in Dulbecco's modified Eagle's medium to fucosylated and nonfucosylated cells, they were seeded to each well. After 4 hr, the supernatant was discarded and the formazan crystals were solubilized in dimethyl sulfoxide (Sigma). The converted dye's optical density was determined by absorbance measurement at 570 nm in a microplate reader (Thermo Fisher Scientific).

2.8 | Colony-forming unit assay

The colonogenicity property of fucosylated and nonfucosylated UCB-CD133+ cells seeded on the 2D- and 3D-like culture systems at the baseline (Day 0) and on Day 7 was investigated by using Methocult media H4435 (Stem Cell Technologies). Therefore, 5×10^3 cells were seeded on the selectin-coated scaffold, the PLLA scaffold, and the 2D culture system, resuspended in Methocult, and then were seeded in triplicates onto 35 mm plates (Grainger). As the cells hardly adhered to the scaffold, after a 7 day period, they were washed several times to separate and then cultured at 37°C, 5% CO_2, and 20% O_2 for 14 days, in accordance with the manufacturer's guidelines. The colonies were then manually numerated to identify colony-forming unit–granulocyte (CFU–G), CFU–macrophage (CFU–M), CFU–granulocyte

TABLE 1 CXCR4 and GAPDH genes primer sequence

Gene (accession no.)	Primer sequences
CXCR4-F	TGAACCCCATCCTCTATGCTT
CXCR4-R	GATGAATGTCCACCTCGCTTT
GAPDH-F	TGCACCACCAACTGCTTAGC
GAPDH-R	GGCATGGACTGTGGTCATGAG

Abbreviations: CXCR4, C-X-C chemokine receptor type 4; F, forward; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; R, reverse.

macrophage (CFU–GM), and CFU–granulocyte, erythrocyte, macrophage, megakaryocyte (CFU–GEMM).

2.9 | Scanning electron microscopy

The uHSCs–PLLA constructs were fixed in 2.5% glutaraldehyde. Then, to get dehydrated, the scaffolds were put into alcohol concentration gradients and dried. Finally, they were installed onto aluminum stubs, and sputter coated with gold. The samples were investigated using scanning electron microscopy (SEM; S-4500; Hitachi) at an accelerating voltage of 20 kV.

2.10 | Homing gene expression

Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to study the expression of CXCR4 gene and evaluate the adhesion ability of expanded fucosylated and nonfucosylated UCB-CD133+ cells on nanofiber scaffolds and 2D culture system for 7 days. The total RNA was extracted by TRIzol (Sigma) protocol, as 1 ml of Trizol was subjected with every 10^6 cells. After adding chloroform, the samples were centrifuged. The supernatant was discarded and isopropanol was added on the aqueous phase and only the RNA plate was rinsed with 75% ethanol. The Thermo Fisher Scientific kit was applied to synthesize the complementary DNA (cDNA). Then, cDNA was mixed well with real-time master mix (Ampilicon), gene-specific primers, and glyceraldehyde 3-phosphate dehydrogenase control gene were used to perform RT-PCR (ABI), too. All reactions were done in triplicate. The primer sequences are shown in Table 1.

2.11 | Immunofluorescence assay

At first, PBS containing 4% (wt/vol) paraformaldehyde (Sigma) was used to fix the uHSCs expanded in all groups for 20 min at 4°C. Then the cells were processed for immunofluorescence staining. Nonspecific binding of the antibodies was blocked by uHSC's incubation with 5% goat serum in PBS for 60 min at RT. Then, overnight incubation of cells at 4°C with rabbit anti-human CXCR4 primary antibodies (Abcam) was done. Afterward, the cells were incubated with a conjugated secondary antibody (Abcam) for 90 min at RT and then they were washed with PBS. Nucleus staining in ICC analysis was performed using 4′,6-diamidino-2-phenylindole. Images were taken by a phase contrast fluorescent microscope (Nikon).

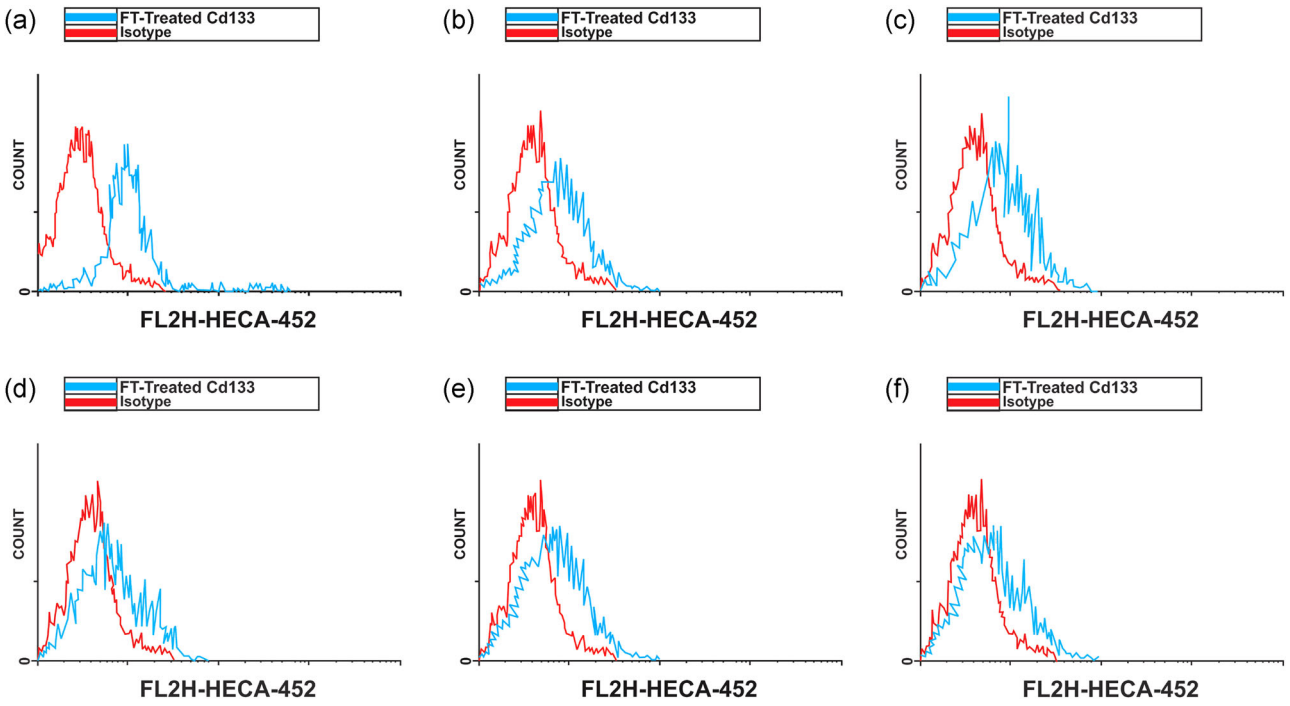

FIGURE 1 Flow cytometric analysis showed (a) the purity of fucosylated CD133+ cells before incubation; (b) the purity of fucosylated CD133+ cells after 7 days expansion on PLLA scaffold; (c) the selectin-coated scaffold; (d) the purity of nonfucosylated CD133+ after 7 day expansion incubation on PLLA scaffold; (e) the selectin-coated scaffold; and (f) the purity of fucosylated and nonfucosylated CD133+ cells after 7 day expansion incubation on 2D culture system. 2D, two-dimensional; PLLA, poly L-lactic acid [Color figure can be viewed at wileyonlinelibrary.com]

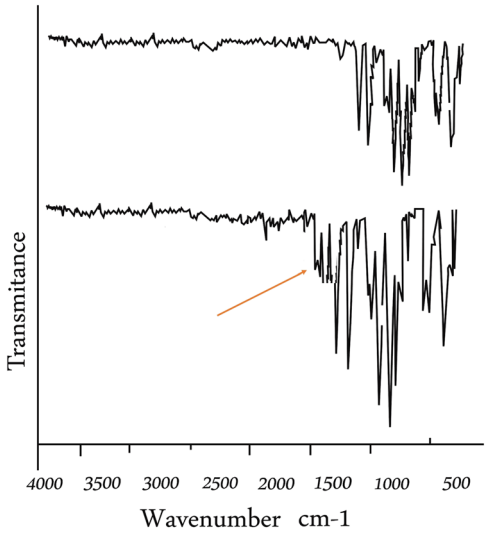

FIGURE 2 ATR–FTIR spectroscopy of PLLA nanofiber and selectin-coated PLLA (The arrow shows the amid group of the selectin). ATR–FTIR, attenuated total reflection–Fourier-transformation infrared technique; PLLA, poly L-lactic acid [Color figure can be viewed at wileyonlinelibrary.com]

2.12 | Statistical analysis

To analyze the results of this study, one-way analysis of variance and Bonferroni's post hoc test were used to compare the selectin coated PLLA scaffols, and PLLA scaffold with 2D by Graphpad Prism 6 software (Graphpad Software Inc.) and the significance level was assumed at $p < .05$.

3 | RESULTS

3.1 | Flow cytometry analysis

As seen in Figure 1a, flow cytometry analysis of CD133 surface markers in fucosylated cells revealed 89% purity, before expansion. The purity of other groups after 7-day incubation is shown in Figure 1b–f: fucosylated CD133+ cells on selectin-coated scaffold (60% purity), fucosylated cells on PLLA scaffold (48% purity), nonfucosylayed cells on selectin-coated scaffold (46% purity), and nonfucosylayed cells on PLLA scaffold (43% purity).

Fucosylated and nonfucosylated CD133+ cells in 2D culture system showed the same purity (33% purity).

3.2 | Modification and characterization

The hydrophilicity and grafting ability of selectin onto the PLLA scaffold surface were determined using ATR–FTIR spectroscopy. The IR spectrum of selectinated PLLA compared with the PLLA showed novel peaks, which are typically related to amide-type 1 (1,500–1,600 cm^{-1}) regarding selectin (Figure 2).

3.3 | 3-(4,5-Dimethyl-thiazolyl-2)-2, 5-diphenoltetrazolium bromide assay

To evaluate the biocompatibility of the fabricated scaffold and proliferation of the fucosylated and nonfucosylated CD133+ cells in the 2D culture system and the PLLA scaffold, MTT assay was performed 1, 3, 5, and 7 days after cell seeding. Nonfucosylated cells on 2D cell culture were applied as a control group. It is illustrated in Figure 3 that the viability of fucosylated cells on selectin scaffold was notably more than the other groups during the culture period.

3.4 | Colony formation of expanded UCB-CD133+ cells

The results of CFU assay in expanded cells on 3D scaffolds at Day 7 showed the clonogenic potential of fucosylated and nonfucosylated CD133+ cells. Figure 4a,b shows CFU–GEMM, CFU–GM, CFU–G, and CFU–M counts in fucosylated and nonfucosylated cells expanded on 2D culture system, PLLA scaffold and selectin-coated PLLA scaffold, respectively. Statistically significant differences were found in the counts of CFU–GEMM and CFU–GM colonies in fucosylated cells expanded on selectin-coated scaffolds ($p < .05$), but statistically significant differences were not observed in the counts of CFU–G and CFU–M colonies between all 3D scaffolds ($p < .05$; Figure 5a–e). It should be noted that there was a higher total colony count in the 3D scaffold compared with the 2D culture systems and the total colony counts were higher in fucosylated cells cultured on selectin-coated scaffold compared with other groups ($p < .05$).

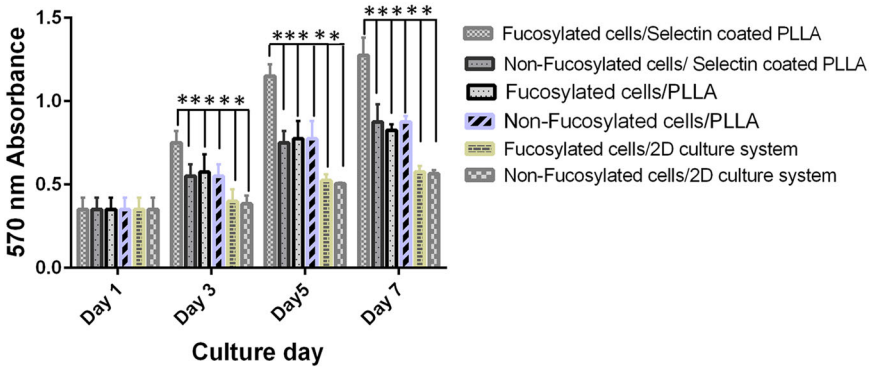

FIGURE 3 Viability of fucosylated and nonfucosylated cells expanded on 2D and selectin-coated scaffold during 1, 3, 5, and 7 days culture period. Results are presented as mean ± *SD*. 2D, two-dimensional; PLLA, poly L-lactic acid; *SD*, standard deviation. Significant levels are $^{*}p < .05$ [Color figure can be viewed at wileyonlinelibrary.com]

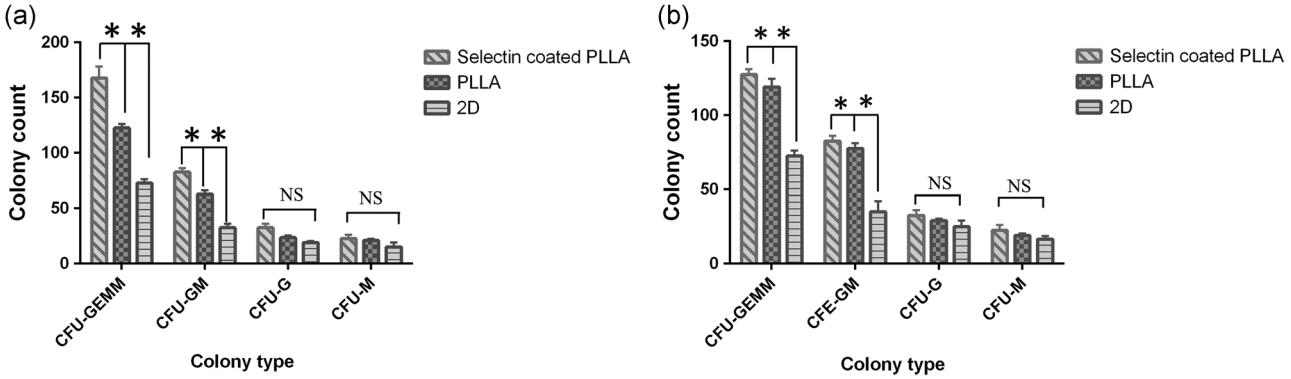

FIGURE 4 CFU assay of total colony numbers in (a) fucosylated and (b) nonfucosylated groups. 2D, two-dimensional; CFU–G, colony-forming unit–granulocyte; CFU–GEMM, colony-forming unit–granulocyte, erythrocyte, macrophage, megakaryocyte; CFU–GM, colony-forming unit–granulocyte macrophage; NS, not significant; PLLA, poly L-lactic acid; *SD*, standard deviation. Significant levels are $^{*}p < .05$

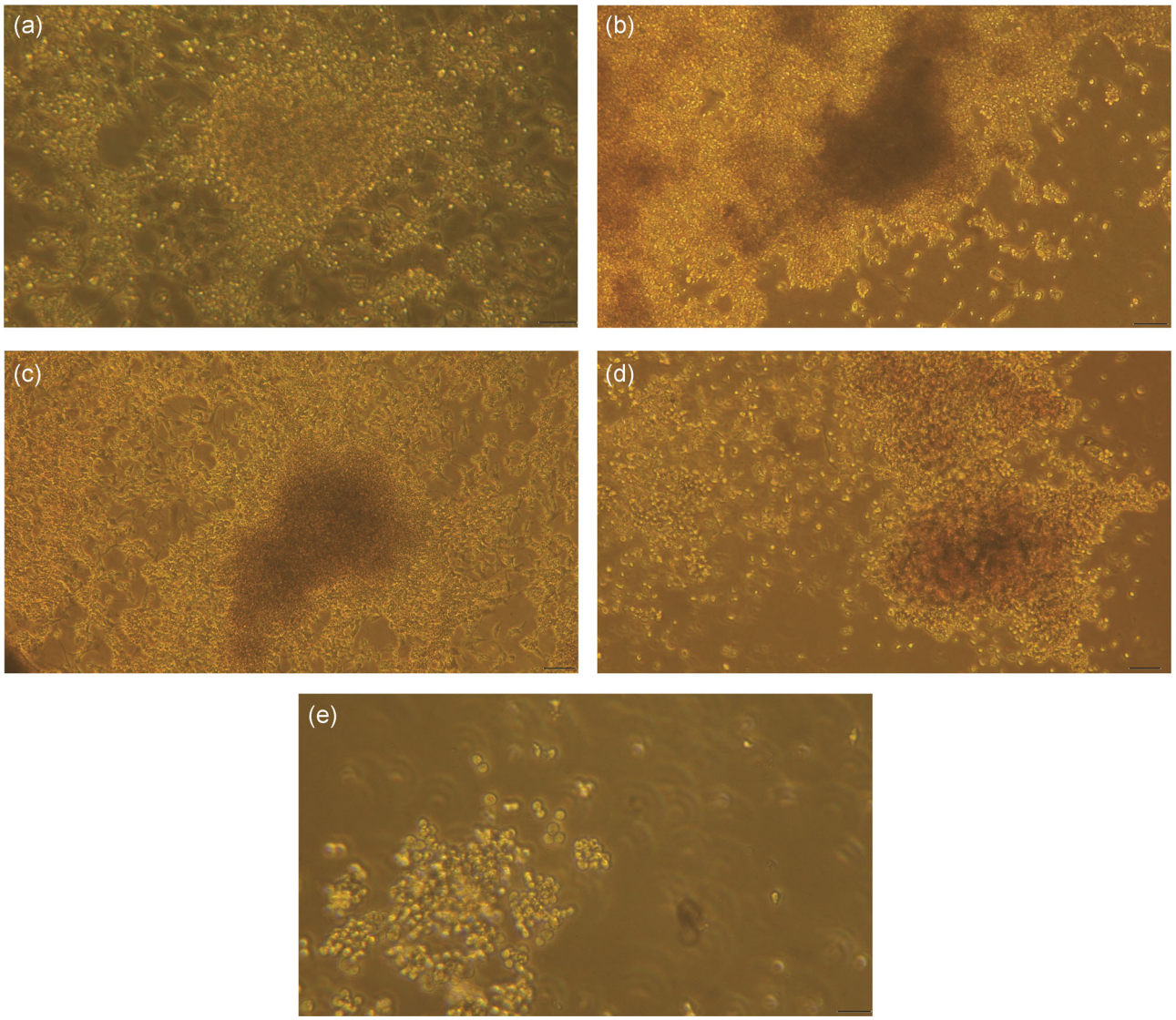

FIGURE 5 CFU colonies (CFU–GM, CFU–GM, CFU–G, and CFU–GEMM) based on the total CFU analyzed for Day 7 cells expanded after 14 days. (a) Nonfucosylated cells on PLLA scaffold. (b) Fucosylated cells on PLLA scaffold. (c) Nonfucosylated cells on selectin-coated PLLA scaffold. (d) Fucosylated cells on selectin-coated PLLA scaffold. (e) Fucosylated and nonfucosylated cells on 2D culture system. Scale bars = 100 μm. 2D, two-dimensional; CFU–G, colony-forming unit–granulocyte; CFU–GEMM, colony-forming unit–granulocyte, erythrocyte, macrophage, megakaryocyte; CFU–GM, colony-forming unit–granulocyte macrophage; PLLA, poly L-lactic acid [Color figure can be viewed at wileyonlinelibrary.com]

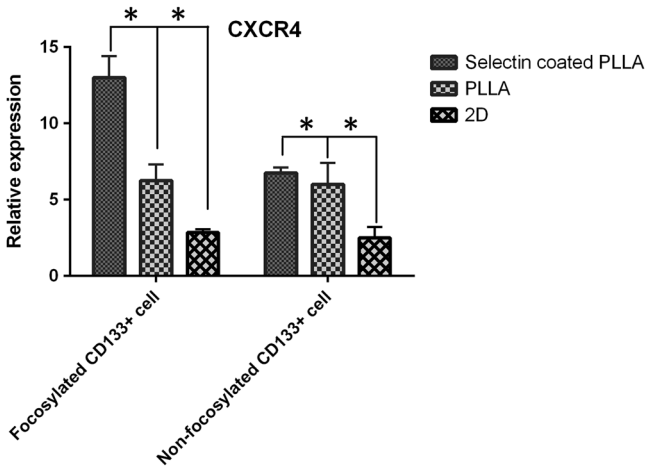

FIGURE 6 Expression of CXCR4 gene in fucosylated and nonfucosylated cells expanded on 2D culture system, PLLA scaffold and selectin-coated PLLA. Real-time PCR showed a significant increase in expression of CXCR4 gene on selectin-coated PLLA scaffolds on Day 7. 2D, two-dimensional; PCR, polymerase chain reaction; PLLA, poly L-lactic acid. Significant level is $^{*}p < .05$

3.5 | Evaluation of CXCR4 expression

To assess fucosylated and nonfucosylated cells homing, the CXCR4 expression level was evaluated on Day 7, using RT-PCR. As shown in Figure 6, low expression of CXCR4 was detected in nonfucosylated cells cultured on a 2D culture system. We observed a higher level of CXCR4 gene expression in fucosylated cells expanded on selectin-coated scaffold compared with the other groups. The relative expression of the CXCR4 gene in the messenger RNA (mRNA) levels in Day 7 is depicted in Figure 6. Multi-fold increase in nonfucosylated and fucosylated CD133+ cells expanded on a 2D culture system (2.3- and 2.4-fold), a PLLA scaffold (6.7- and 6.9-fold), and a selectin-coated scaffold (7.3- and 12.8-fold) were observed compared with the control group (nonfucosylated cells on 2D culture system on Day 0). As a result, the CXCR4 expression was upregulated in fucosylated CD133+ cells when cultured on selectin-coated PLLA scaffolds.

3.6 | Scanning electron microscopy assay

Attachment of fucosylated and nonfucosylated CD133+ cells to the selectin-coated PLLA scaffold and the PLLA scaffold was evaluated by SEM imaging after 7 days. The results depicted the amount of growth and scaffold attachment in the above-mentioned cells (Figure 7a–e).

3.7 | Immunofluorescence staining

To evaluate uHSCs homing on the 3D-like scaffold, the expression of CXCR4 was shown in the immunostaining assay (Figure 8). In fucosylated and nonfucosylated HSCs expanded on the 2D culture system, weak immune reactivity of CXCR4 was observed on Day 7. Fucosylated cells expanded on PLLA scaffold, nonfucosylated cells expanded on selectin-coated PLLA and PLLA scaffold showed moderate immune reactivity of CXCR4. In fucosylated cells expanded on selectin-coated PLLA scaffold, strong immune reactivity was observed in the CXCR4 surface marker on Day 7. To analyze secondary control of uHSCs, they were just incubated with a secondary antibody in the absence of primary staining. These data are displayed in Figure 8.

4 | DISCUSSION

Recently, tissue engineering has found special importance in medical sciences and many investigations have focused on it. In accordance with the healing potency of tissue engineering, some diseases have been treated as well. In this regard, different nanofibrous scaffolds have been used to improve the efficiency of cell culture and tissue engineering of stem cells in a 3D manner. The improved capacity of the PLLA scaffolds with a high surface-to-volume ratio, as an environment for uHSCs culture, led to the employment of these nanostructures in the present study (Islami, Mortazavi, Soleimani, & Nadri, 2018; Mirzaei et al., 2019; Nie et al., 2008).

Given the features such as easy access and lack of immune response in the uHSCs transplantation, UCB could be used as a traditional substitute donor source of HSCs. But with regard to the uHSC's low number and some defects in homing to the hematopoietic niche, fucosylation, and expansion of these cells on nanofiber scaffold were considered.

Because fucosylation of uHSCs was accepted as a significant strategy for increasing of PSGL binding to P-selectin (Xia, McDaniel, Yago, Doeden, & McEver, 2004), its importance in uHSC's homing to scaffolds was confirmed by gene expression and protein expression analyses of the CXCR4 too. Furthermore, the results of this study corresponded with the findings of the previous studies about CXCR4 importance in the homing process. Wyszynski et al. (2005) mentioned that CXCR4 played an essential role in homing and transplantation. Iwasaki (2010) stated that CXCR4 was a vital element in self-renewal, survival, and motility. Nie et al. (2008) demonstrated that CXCR4 was a key marker in HSC proliferation, their implantation and homing in proper niche.

In the present study, the results of the biocompatibility of the PLLA scaffold and selectin-coated PLLA scaffold by MTT assay indicated that fucosylated cells expanded on the selectin-coated PLLA scaffold were significantly more viable than the PLLA and 2D culture system. These results could be the consequence of two main factors: (a) 3D like structure of PLLA scaffold provided a suitable surface area with the higher surface-to-volume ratio for cell seeding and mimicking cell's natural microenvironment; and (b) the presence of selectin as a receptor for PSGL ligand on PLLA scaffold surface improved cell proliferation and viability in comparison with the 2D culture system and nonfucosylated cells (Dubey & Mequanint, 2011; Popat et al., 2015).

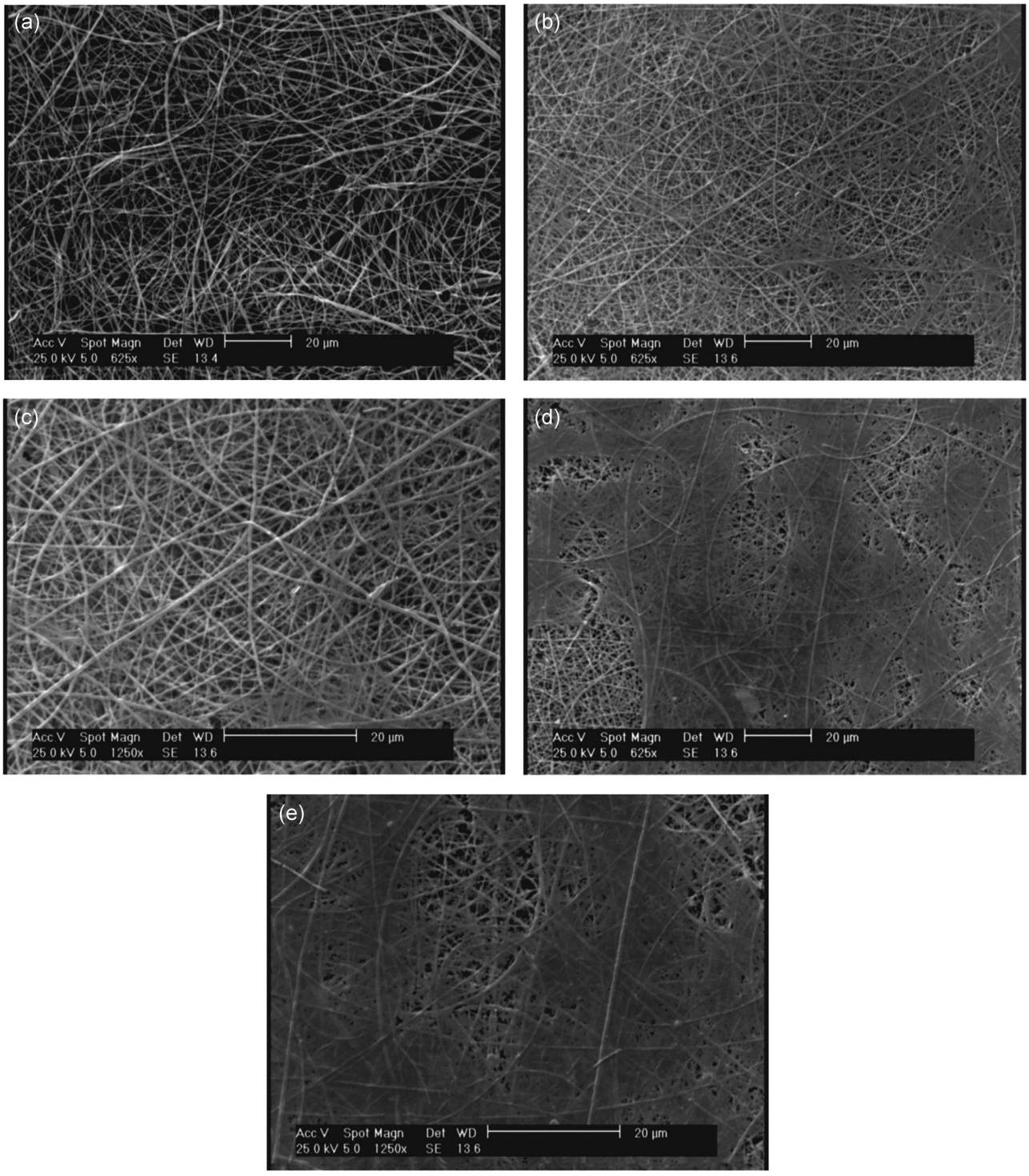

FIGURE 7 (a) SEM imaging. Morphology of fabricated PLLA scaffold. (b) SEM micrograph of nonfucosylated cells expanded on PLLA scaffold. (c) Fucosylated cells expanded on PLLA scaffold. (d) Nonfucosylated cells expanded on selectin-coated PLLA scaffold. (e) Fucosylated cells expanded on selectin-coated PLLA scaffold. PLLA, poly L-lactic acid; SEM, scanning electron microscopy

Moreover, in this study, we observed more CFU–GEMM colonies in fucosylated CD133+ cells expanded on the selectin-coated scaffold than CFU–GM compared with other groups, indicating less differentiation of these cells. Furthermore, the highest number of the colony in fucosylated CD133+ cells, in the presence of the selectin, highlighted the relationship of the selectin and fucosylation. A 3D-like culture system provided improved artificial niche, leading to a higher rate of the colony formation in expanded uHSCs compared with the 2D culture system. The presence of selectin and its interactions with artificial niche have been shown to optimize uHSCs function, proliferation, and survival. It should be mentioned that the sizes of the colonies were not significantly different in either

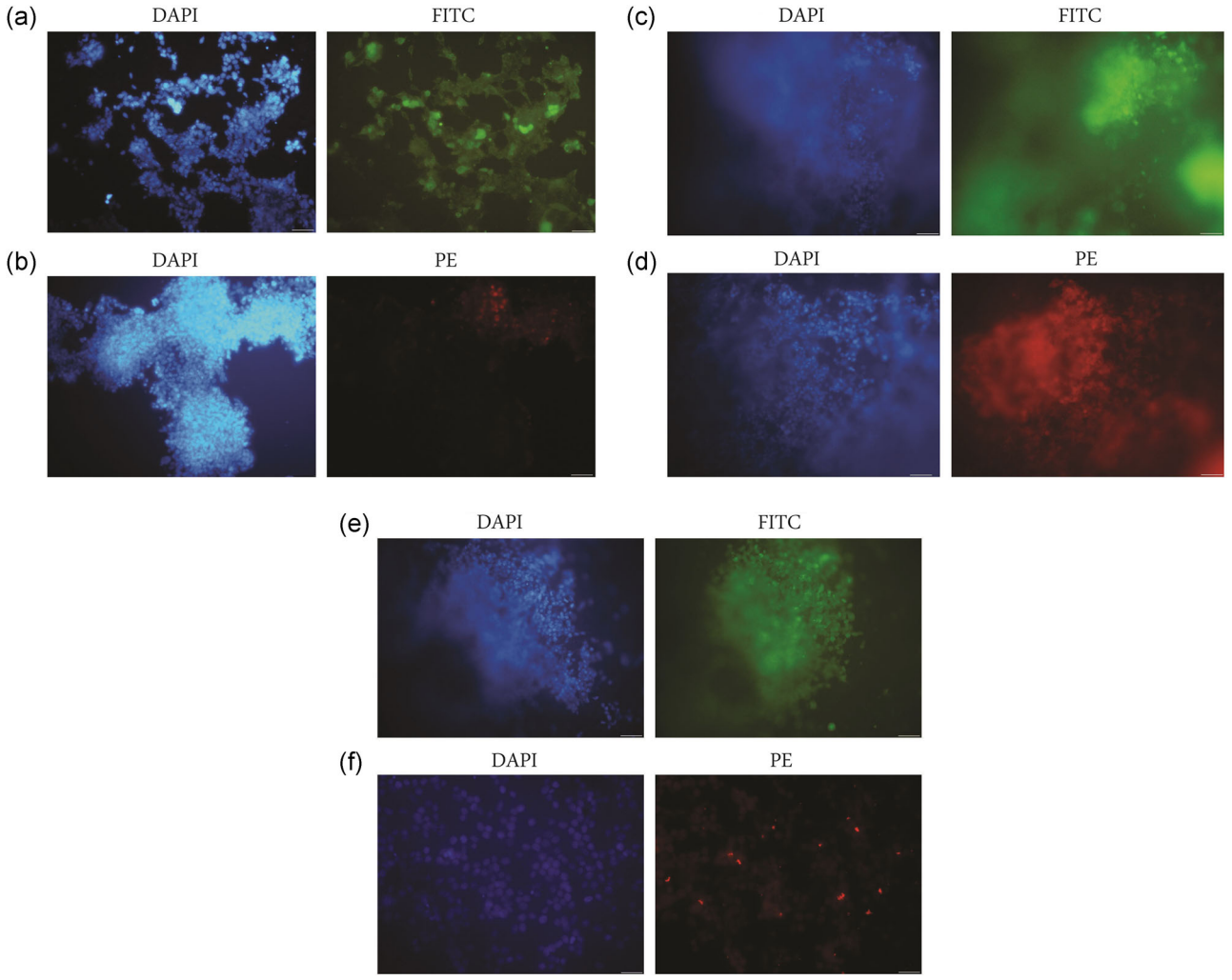

FIGURE 8 In vitro expression analysis of CXCR4 marker after 7 days. (a) Nonfucosylated cells expanded on 2D culture system. (b) Fucosylated cells expanded on 2D culture system. (c) Nonfucosylated cells expanded on PLLA scaffold. (d) Fucosylated cells expanded on PLLA scaffold. (e) Nonfucosylated cells expanded on selectin-coated PLLA scaffold. (f) Fucosylated cells expanded on selectin-coated PLLA scaffold. Scale bars = 100 μm. Staining of nucleus was performed by DAPI (blue). 2D, two-dimensional; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PLLA, poly L-lactic acid. *Source*: For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article [Color figure can be viewed at wileyonlinelibrary.com]

fucosylated or nonfucosylated groups, indicating that short term fucosylation had no changes in the growth rates of the UCB–HSCs (Figure 5).

Taken together, the obtained results of MTT assay, CFU assay and CXCR4 gene expression at mRNA and protein levels demonstrated that the mimicking of the native niche by 3D like PLLA scaffold or artificial niche has an important role in enhancing the expansion efficiency and viability of uHSCs. Also, the results of ICC assay in the 3D-like culture showed a strong immune reactivity in CXCR4 protein expression in fucosylated CD133+ cells expanded on selectin-coated scaffold compared with the other groups. Furthermore, comparison between 2D and 3D like culture, fucosylated and nonfucosylated cells, illustrated that fucosylated cells expanded on PLLA and 2D culture system, nonfucosylated cells expanded on selectin-coated PLLA and PLLA scaffold and 2D culture system were not successful in optimal homing because of nonfunctional and immature binding between PSGL and selectin. Conversely, due to synergic effect of fucosylation, 3D like culture system and selectin coating, in fucosylated cells expanded on selectin-coated PLLA scaffolds, we observed the highest homing that implied these element were key factors in HSC's homing (Darvish et al., 2019; Islami et al., 2018).

5 | CONCLUSIONS

On the basis of this study results, selectin-coated PLLA scaffolds could to be used for hematological tissue engineering and regenerative medicine using human-fucosylated cells. So, this strategy may be considered as a promising candidate for the treatment of adult patients with hematological disorders in future. However, more investigations can permit this approach particularly in the in vivo investigations.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

M. I., Y. M., and S. N. conceived and designed this study. Z. P. and F. S. performed and analyzed biological experiments. E. S. and E. D. A. performed and analyzed engineering experiments and wrote the first draft of the manuscript. M. K. performed the immunofluorescence staining in the transplanted hematopoietic stem cells. M. D. performed the analyzed experiments and discussed the results. M. I. analyzed the experiments, discussed the results, wrote the paper, and approved the manuscript too. All authors approved the final manuscript.

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