Influence of SPIO labelling on the function of BMSCs in chemokine receptors expression and chemotaxis

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Bone marrow-derived mesenchymal stem cells (BMSCs) are increasingly being used in bone marrow transplantation (BMT) to enable homing of the allogeneic hematopoietic stem cells and suppress acute graft versus host disease (aGVHD). The aim of this study was to optimize the labelling of BMSCs with superparamagnetic iron oxide particles (SPIOs), and evaluate the impact of the SPIOs on the biological characteristics, gene expression profile and chemotaxis function of the BMSCs. The viability and proliferation rates of the SPIO-labeled BMSCs were analyzed by trypan blue staining and CCK-8 assay respectively, and the chemotaxis function was evaluated by the transwell assay. The expression levels of chemokine receptors were measured by RT-PCR and flow cytometry. The SPIOs had no effect on the viability of the BMSCs regardless of the labelling concentration and culture duration. The labelling rate of the cells was higher when cultured for 48h with the SPIOs. Furthermore, cells labeled with 25μg/ml SPIOs for 48h had the highest proliferation rates, along with increased expression of chemokine receptor genes and proteins. However, there was no significant difference between the chemotaxis function of the labeled and unlabeled BMSCs. To summarize, labelling BMSCs with 25μg/ml SPIOs for 48h did not affect their biological characteristics and chemotaxis function, which can be of significance for *in vivo* applications.

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Abstract

Bone marrow-derived mesenchymal stem cells (BMSCs) are increasingly being used in bone marrow transplantation (BMT) to enable homing of the allogeneic hematopoietic stem cells and

suppress acute graft versus host disease (aGVHD). The aim of this study was to optimize the labelling of BMSCs with superparamagnetic iron oxide particles (SPIOs), and evaluate the impact of the SPIOs on the biological characteristics, gene expression profile and chemotaxis function of the BMSCs. The viability and proliferation rates of the SPIO-labeled BMSCs were analyzed by trypan blue staining and CCK-8 assay respectively, and the chemotaxis function was evaluated by the transwell assay. The expression levels of chemokine receptors were measured by RT-PCR and flow cytometry. The SPIOs had no effect on the viability of the BMSCs regardless of the labelling concentration and culture duration. The labelling rate of the cells was higher when cultured for 48h with the SPIOs. Furthermore, cells labeled with 25μg/ml SPIOs for 48h had the highest proliferation rates, along with increased expression of chemokine receptor genes and proteins. However, there was no significant difference between the chemotaxis function of the labeled and unlabeled BMSCs. To summarize, labelling BMSCs with 25μg/ml SPIOs for 48h did not affect their biological characteristics and chemotaxis function, which can be of significance for *in vivo* applications.

Key words: SPIO, mesenchymal stem cell, chemokine receptor, chemotaxis, aGVHD.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is known to improve the outcomes of patients with hematological malignancies [1,2]. However, it is beset with several challenges such as the lack of suitable donors [3,4] and induction of acute graft versus host disease

(aGVHD) [5,6]. Transplantation of *ex vivo*-expanded bone marrow mesenchymal stem cells (BMSCs) [7,8] can obviate the disadvantages of allo-HSCT. BMSCs were first identified by Friedenstein as fibroblast colonies formed by bone marrow explants [9]. Recent studies show that BMSCs can promote the homing of transplanted bone marrow cells through specific chemokines and their receptors, and augment hematopoiesis by secreting multiple cytokines [10-15]. Moreover, the BMSCs have been shown to mitigate severe, steroid-refractory aGVHD in the liver, skin and lungs, although the exact mechanisms are unclear [16-19]. Real-time tracking of the BMSCs after intravenous injection can provide insights into their in situ functions. For instance, BMSCs labeled with superparamagnetic iron oxide particles (SPIOs) can be monitored *in vivo* by non-invasive magnetic resonance imaging (MRI). SPIOs have the advantages of non-toxicity, prolonged retention and stable excretion [20-22]. Most studies on labelling BMSCs have been focused on determining the toxicity of the labelling particles and optimizing the concentration for real-time *in vivo* imaging [23-25]. Therefore, little is known regarding the influence of labelling particles on the chemokine gene expression and chemotaxis function of BMSCs, which is critical to their clinical role in allo-HSCT and aGVHD.

The aim of our study was to determine the impact of different concentrations and labelling durations of SPIOs on the biological characteristics of the BMSCs. We chose rBMSCs from SD rats for our study, which the physiological characteristics meet the requirements of experimental purposes and the needs of subsequent animal experiments. We found that SPIOs can effectively label rBMSCs without affecting their viability and chemotactic functions. The SPIOs-labeled BMSCs can be used to elucidate the mechanisms underlying their functions in BMT and aGVHD

through real-time *in vivo* monitoring.

Material and methods

Isolation and culture of rat BMSCs (rBMSCs)

SD male rats aged 4-5 weeks old and weighing about 160-200g obtained from Animal Experiment Center of Southern Medical University were euthanized by intravenous injection and dissected to expose the femur, and the study was approved by the Institutional Animal Care and Use Committee of Guangzhou First People's Hospital. The euthanizing criteria was established according to former experiment. The ends of the femurs were cut with a pair of bone forceps, and the bone marrow was flushed out using a 27-gauge needle attached to a 10 ml syringe containing alpha-DMEM/F12 (1:1) medium. The single cell suspension was seeded in a 25 cm^2 flask and cultured in alpha-DMEM/F12 supplemented with 10% fetal bovine serum (FBS; Ausgenex, FBS500) and 1% penicillin/streptomycin at 37°C under 5% CO_2 in a humid incubator (Thermo Co.). The culture medium and the non-adherent cells were gently removed 24h later, and fresh medium was added. Thereafter, the cell medium was changed every 2-3 days and the cells were passaged at 90% confluency. The primary isolated rBMSCs were defined as passage 0. Cells from passages < 10 were used for the experiments. The methodology is outlined in Figure 1.

The rBMSCs were cultured with complete alpha-DMEM/F12 with 0, 25, 50 or 75μg/ml SPIOs for 24, 48 or 72h. The expanded BMSCs were identified by flowcytometry and the labelling rate,

proliferation and viability were measured using suitable assays. The expression of different chemokine receptors was analyzed by RT-PCR and flow cytometry. were used to measure the expression of chemokine receptors. Transwell assay was used to evaluate the migration rates of the BMSCs.

Flow cytometry

The primary rBMSCs were washed twice with PBS, re-suspended at the density of $1*10^6$/ml, and incubated with : anti-CD29 (Biolegend Cat # 102221, RRID: AB_528789), anti-CD34 (Santa Cruz Cat # sc-7324, RRID: AB_2291280), anti-CD44 (BD Cat # 553456, RRID: AB_10515282) and anti-CD45 (Biolegend Cat # 202216, RRID: AB_1236411) antibodies at 37°C in the dark for 30 min. The stained cells were washed with PBS and re-suspendered in 200µl PBS(n=3). The rBMSCs co-cultured with SPIOs for 24h, 48h and 72h were trypsinized and centrifuged at 1500 rpm for 15 minutes, and re-suspended in PBS at the density of $1*10^6$ cells/tube. The aliquots were incubated with anti-CXCR-4 (Santa Cruz cat. no. sc-53534, RRID: AB_782002), anti-CXCR-7 (R&D Cat # FAB8399RG, RRID: AB_2917964), anti-CCR-10 (R&D Cat # FAB2815A-025, RRID: AB_1151964) and anti-CXCR-3 (R&D Cat # FAB8109P, RRID: AB_2917963) antibodies for 30 minutes at room temperature in the dark. The stained cells were washed and re-suspended in 200µl PBS. Unstained controls were also included. The cells were acquired in the BD FACSCANTO Plus 10C flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) (n=7), and the expression of the markers was analyzed by Flowjo software (version Flow Jo X 10.0.7r2; FlowJo LLC, Ashland, OR, USA).

106

107 **Cell labelling assay**

108 The rBMSCs were cultured till 50% confluent, and incubated with different concentrations (25,
109 50, 75 and 100μg/ml) SPIOs (Aladdin, cat. no. I140464) for 24 hours. The medium was
110 discarded and fresh medium lacking SPIOs was added. After culturing for another 24h, 48h and
111 72h, the labeled cells were harvested for subsequent tests(n=3).

112

113 **Prussian blue staining**

114 The labeled rBMSCs was washed thrice with PBS and fixed with 4% paraformaldehyde for 30
115 minutes. After discarding the paraformaldehyde, the cells were incubated with 2% potassium
116 ferrocyanide in 6% hydrogen acid at room temperature for 30 minutes. The stained cells were
117 washed thrice with Hank's buffer, counterstained with nuclear fast red for 2-5 minutes, and
118 washed again. The number of positively-stained cells were counted under a microscope at 40x
119 magnification, and the percentage of SPIOs-labeled cells to the total number of cells was
120 calculated(n=3).

121

122 **Trypan blue staining**

123 Labeled rBMSCs were suspended in PBS at the density of $1*10^6$ cells/ml, and 90μl cell
124 suspension was mixed with 10μl 0.4% trypan blue solution. The cells were counted within 3
125 minutes of staining, and the viability was calculated as: number of unstained cells/total
126 cells*100%(n=3).

127

128 **CCK-8 assay**

129 The rBMSCs were seeded in 96-well plates at the density of $2*10^4$/well and cultured overnight.
130 The medium was discarded the following day, and fresh medium containing different
131 concentrations of SPIOs was added. After culturing for 24h, 48h and 72h, the medium was
132 replaced with 100μl fresh medium. CCK-8 reagent was added to each well and the cells were
133 incubated for 2h in the dark. The absorbance (OD) value at 490 nm was measured using a
134 microplate reader (BIO-TEK INSTRUMENTS, INC) (n=5).

135

136 **Quantitative reverse transcription-polymerase chain reaction**

137 The total RNA was extracted using TRIZOL (Thermo, cat. no. 15596-026) according to the
138 manufacturer's instructions, and reverse transcribed to cDNA using PrimerScript RT Master Mix
139 (Takara, Tokyo, Japan). RT-PCR was performed on the IQ5 System (Bio-Rad) using SYBR
140 Green Mastermix (Takara, Tokyo, Japan) (n=3). Each sample was analyzed in duplicate and the
141 relative expression of the target genes was normalized to beta-actin. The primer sequences are
142 listed in Supplementary Table 1.

143

144 **Transwell assay**

145 Complete DMEM/F12/F12 (10% FBS) was dispensed into transwell inserts with pore size of
146 8μm (Corning cat. no. 3422, Costar, Cambridge, MA, USA) in 24-well plates, and incubated
147 overnight. After discarding the medium, control or SPIO-labeled rBMSCs were seeded into the

upper chamber of the inserts in complete medium at the density of $8*10^4$ cells/200µl, with or without chemokine receptor antagonists TAK799, Maraviroc or LY294002. The lower chambers were filled with medium supplemented with the corresponding chemokines i.e., CXCL-10, CCL-4 and CCL-19. After incubating the cells for 48h, the inserts were removed and the cells remaining on the upper surfaces of the membrane were scraped off. The membranes were then washed twice with PBS, fixed in 4% paraformaldehyde for 10 minutes, and stained with 0.5% crystal violet for 5 minutes. The migrated cells on the lower surface of the membranes were counted in three random fields per well under a microscope at low magnification(n=3).

Statistical analysis

Statistical product and service solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for the statistical analysis. The enumeration data were expressed as mean ± standard deviation. Multiple groups were compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, or rank sum test followed by Kruskal-Wallis post hoc test. $P<0.05$ was considered statistically significant.

Results

Isolation and characterization of rBMSCs

The primary rBMSCs were adherent after overnight incubation, and consistently exhibited a uniform fibroblast-like appearance from passage 0 (Figure 2A and B) to passage 10 (Figure 2C and D). As shown in Figure 3, the isolated rBMSCs were CD29+ (96.8%), CD44+ (99.3%),

CD34- (99.2%) and CD45- (98.7%). Based on previous reports, our results suggested that the isolated and expanded cells were rBMSCs.

The BMSCs displayed a fibroblast-like appearance throughout culture. Images show BMSCs from passages (A, C) 0 and (B, D) 10 at 50X and 100X magnification as indicated.

Flow cytometry plots showing percentages of (A) CD29+, (B) CD44+, (C) CD34- and (D) CD45- cells.

rBMScs were optimally labeled with SPIOs after 48h incubation

The rBMSCs were incubated with different concentrations of SPIOs for varying durations, and the presence of SPIOs was detected by Prussian blue staining. As shown in Figure 4A, cells incubated with SPIOs had dense blue-stained iron particles in their cytoplasm and the intensity of the color deepened with increasing concentrations of SPIOs. In contrast, no blue-stained iron particles were observed in the control group (Figure 4A). Furthermore, the percentage of SPIO-labeled cells increased from 54.67%±1.15% after 24h to 98%±1% after 48h of incubation with 50μg/ml SPIOs. The labelling rates of the rBMSCs after 48h incubation with 25, 50 and 75μg/ml SPIOs were 90%±1.73%, 98%±1% and 96%±1% respectively, which were consistently higher than that for the other incubation times. (figure 4B).

SPIOs had no detrimental effect on the viability and proliferation of rBMSCs

The possible effect of the SPIOs on the viability of BMSCs was evaluated by trypan blue dye exclusion test. As shown in Figure 4C and D, trypan blue dye exclusion rates differed from 91%±3.60% to 98.67%±0.58% across the different concentrations of SPIOs, and were not significantly different between the groups. CCK-8 assay further showed that cells labeled with 25μg/ml SPIOs had a higher OD value than the cells incubated with higher doses of SPIOs. In addition, the OD values of cells incubated with SPIOs for 24h and 72h showed no significant difference for the different concentrations (Figure 4E).

(A) Representative images showing Prussian blue-stained iron particles in the BMSCs labeled with different concentrations of SPIOs for varying durations. (B) Labelling rate in the indicated groups. (C) Representative images showing live cells in the indicated groups after trypan blue staining. (D) Viability rates in the indicated groups. (E) Proliferation rates in the indicated groups as measured by CCK-8 test. Data are individual means or the mean ± SD of each group from three separate experiments. *$p<0.05$, **$p<0.01$, ***$p<0.001$

SPIOs-labeled rBMSCs expressed higher levels of chemokine receptors and cytokines

As shown in Figure 5A, the control and 25μg/ml SPIOs-labelled cells expressed consistently high levels of CCR5, CCR10, CXCR3, CXCR5 and IL11 mRNAs after 48h of incubation. Furthermore, cells incubated with 25μg/ml SPIOs for 48h showed significant upregulation of CCR5 and CXCR5 compared to the 72h group for the same concentration ($p<0.05$), and of CCR10 and CXCR3 compared to cell incubated with 50μg/ml SPIOs for 24h. Likewise, IL-6

211 and CXCR7 genes were also upregulated in the 25μg/ml SPIOs/48h group compared to the
212 control and 25μg/ml SPIOs/24h group, and IL-11 was upregulated compared to that in the
213 50μg/ml SPIOs/48h group (Figure 5B).
214 Furthermore, flow cytometry experiments showed that the percentage of CXCR3+ cells in the
215 rBMSCs treated with 25μg/ml SPIOs for 24, 48 and 75h were 26.58%±3.68%, 71.02%±9.93 and
216 58.31%±13.09% respectively, and that of CCR10+ cells at the respective time points were
217 25.43%±9.49%, 43.09%±6.68 and 20.72%±1.89%. The untreated control cells also showed a
218 higher expression of CXCR3 after 48h, whereas the percentage of CCR10+ cells was unaffected
219 by the duration of culture. There was no significant difference in the percentage of CXCR3+ or
220 CCR10+ cells between the control and 25μg/ml SPIOs/48h groups (Figure 6A-C). The
221 percentage of CXCR7+ cells in the control group after 24, 48 and 72h of culture were
222 67.79%±7.77%, 68.59%±6.91 and 53.02%±8.82% respectively, which were significantly higher
223 than in the 25μg/ml SPIOs-labeled group at each time point ($p<0.001$; Figure 5E). Nevertheless,
224 rBMSCs labeled with 25μg/ml SPIOs for 48h had the highest percentage of CXCR7+ cells
225 (30.03%±9.38%) compared to those labeled with higher concentrations of SPIOs for the same
226 duration. No significant difference was observed between the different SPIO-labeled groups
227 incubated for 24h or 72h.
228 The expression level of CXCR4 was the highest amongst all chemokine receptors in both the
229 control and SPIOs-labeled groups. The percentage of CXCR4+ cells in the control and 25μg/ml
230 SPIOs-labeled groups were 94.81%±1.72% and 93.47%±3.41% respectively after 24h of culture,
231 and were higher than that in the 50/75μg/ml SPIOs-labeled groups for the same duration

232 ($p<0.001$). In contrast, the cells incubated with 25μg/ml SPIOs for 48h or 72h had a higher
233 proportion of CXCR4+ cells compared to the control ($p<0.01$ at 48h) and other labeled groups
234 ($p<0.05$ vs 75μg/ml SPIOs-labeled group at 72h).
235
236 **SPIOs did not affect the migration and chemotactic function of rBMSCs**
237 To determine the effect of the SPIOs on the chemotactic migration of rBMSCs *in vitro*, we
238 cultured the control or 25μg/ml SPIOs-labeled cells in a transwell system in the presence or
239 absence of the CXCL10 (TAK 799), CCL4 (MARAVIORIC) and CCL19 (LY294002)
240 antagonists, and added the respective chemokines to the lower chambers (Figure 7A). After 48
241 hours, the number of BMSCs that had migrated to the other side of the transwell membrane was
242 counted in each group. The migration rates of both the control and SPIOs-labeled cells were
243 significantly higher in the absence of the chemokine antagonists ($p<0.001$). The percentage of
244 migrating cells per field in response to CXCL10, CCL4 and CCL19 were 81.67±7.36%,
245 82.00±6% and 69.33±3.05% respectively in the control group, and 74.67±2.51%, 82±2% and
246 /68.33±1.52% in the SPIOs-labeled group. The migration rates of the control group decreased to
247 29.33±4.04%, 18.00±3% and 20.00±4% in the presence of TAK 799, MARAVIORIC and
248 LY294002 respectively, and that of the SPIOs-labeled cells decreased to 32.33±2.08%,
249 27.33±2.51% and 29.67±2.08% (Figure 7B). There was no significant difference in the migration
250 rates of the control and SPIOs-labeled BMSCs in any of the conditions, indicating that the SPIOs
251 had no effect on the migration and chemotactic behavior of the rBMSCs.
252

253 **Discussion**

254 We found that labelling rBMSCs with SPIOs did not affect their viability or chemotactic
255 functions. The cells were optimally labeled when incubated with 25μg/ml SPIOs for 48h, which
256 increased the expression levels of chemokines and chemokine receptors, and therefore
257 maintained their chemotactic migration *in vitro*.

258 A previous study showed that labelling BMSCs with ferucarbotran, a contrast agent consisting of
259 iron oxide microparticles, decreased their migration ability in a dose-dependent manner [11]. We
260 labeled the rBMSCs with a lower concentration of SPIOs to achieve an efficient labelling rate
261 without compromising on cell viability. This is the first study to show that BMSCs incubated
262 with 25μg/ml SPIOs for 48h can be effectively labeled, while maintaining viability and
263 migration ability. This finding is of clinical significance since SPIOs-labeled BMSCs can be
264 tracked *in vivo* to determine their role in the homing of allo-hematopoietic stem cells and the
265 mitigation of aGVHD. The latter is the main life-threatening complication of allo-HSCT. Several
266 clinical and animal model studies have shown that transplantation of BMSCs improve the
267 outcomes of allo-HSCT [5,26,27]. Cynthia et al conducted a meta-analysis and found that
268 allogeneic BMSCs can be used for the prophylaxis and treatment of aGVHD [28]. Therefore, our
269 results can be helpful in elucidating the mechanisms underlying the clinical benefits of BMSCs.

270 The SPIOs-labeled BMSCs expressed higher levels of different chemokines compared to the
271 unlabeled controls. While CXCR4 mRNA was upregulated in the labeled cells, the percentage of
272 cells expressing CXCR4 protein were similar across all groups. This can be attributed to the fact
273 that gene expression is regulated at the transcriptional, mRNA processing and translational

levels, any of which may influence the surface expression of the protein. In addition, the cells labeled with 25ug/ml SPIOs for 48h showed the highest expression of CXCR7 among all groups. However, there was no significant difference between the migration rates of the labeled and unlabeled BMSCs, which suggests that the chemokine receptors affect the migration of BMSCs by varying degrees. Given the higher expression of CXCR4 in the SPIOs-labeled cells compared to the controls, we surmise that it may be an important factor in the chemotactic migration of BMSCs.

Interestingly, the labelling rate was higher in cells incubated with the SPIOs for 48h compared to 24h or 72h. This is likely due to the fact that the number of cells accelerated rapidly during prolonged culture while the amount of SPIOs remained the same. The labeled cells may allocate the SPIOs into their descendant cells for several generations but after a certain threshold, the amount of SPIOs dilutes away. Thus, the number of labeled cells initially increased in a time-dependent manner before decreasing.

There are several limitations in our study that ought to be considered. First, we did not analyze the secreted cytokine profile of the labeled BMSCs, which is of clinical relevance the cytokines secreted by the BMSCs influence hematopoiesis. Therefore, our next objective will be to analyze the mRNA and protein levels of multiple cytokines in the labeled BMSCs. Furthermore, other genes involved in BMSC migration will also have to be analyzed. Finally, the clinical applicability of the SPIOs-labeled BMSCs will have to be validated in an in vivo aGVHD model. To summarize, our findings will help facilitate greater clinical use of Fe nanoparticles.

295 **Conclusions**

296 We have demonstrated for the first time that SPIOs can effectively label rBMSCs without
297 affecting their viability and chemotactic functions. Thus, SPIOs-labeled BMSCs can be used to
298 elucidate the mechanisms underlying their functions in BMT and aGVHD through real-time *in*
299 *vivo* monitoring.

300

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304

305 **Ethical Statement:** The procedure for extracting primary cells from animals was reviewed and
306 approved by the Review Opinions of Laboratory Animal Ethics Committee of Guangdong
307 Pharmaceutical University.

308

309 **Data assess statement:** The data that support the findings of this study are available upon
310 reasonable request from the authors.

311 **Conflict of Interest:** All authors have completed the ICMJE uniform disclosure form . The
312 authors have no conflicts of interest to declare.

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Figure 1

Schematics of the experimental design.

The rBMSCs were cultured with complete alpha-DMEM/F12 with 0, 25, 50 or 75μg/ml SPIOs for 24, 48 or 72h. The expanded BMSCs were identified by flowcytometry and the labelling rate, proliferation and viability were measured using suitable assays. The expression of different chemokine receptors was analyzed by RT-PCR and flow cytometry. were used to measure the expression of chemokine receptors. Transwell assay was used to evaluate the migration rates of the BMSCs.

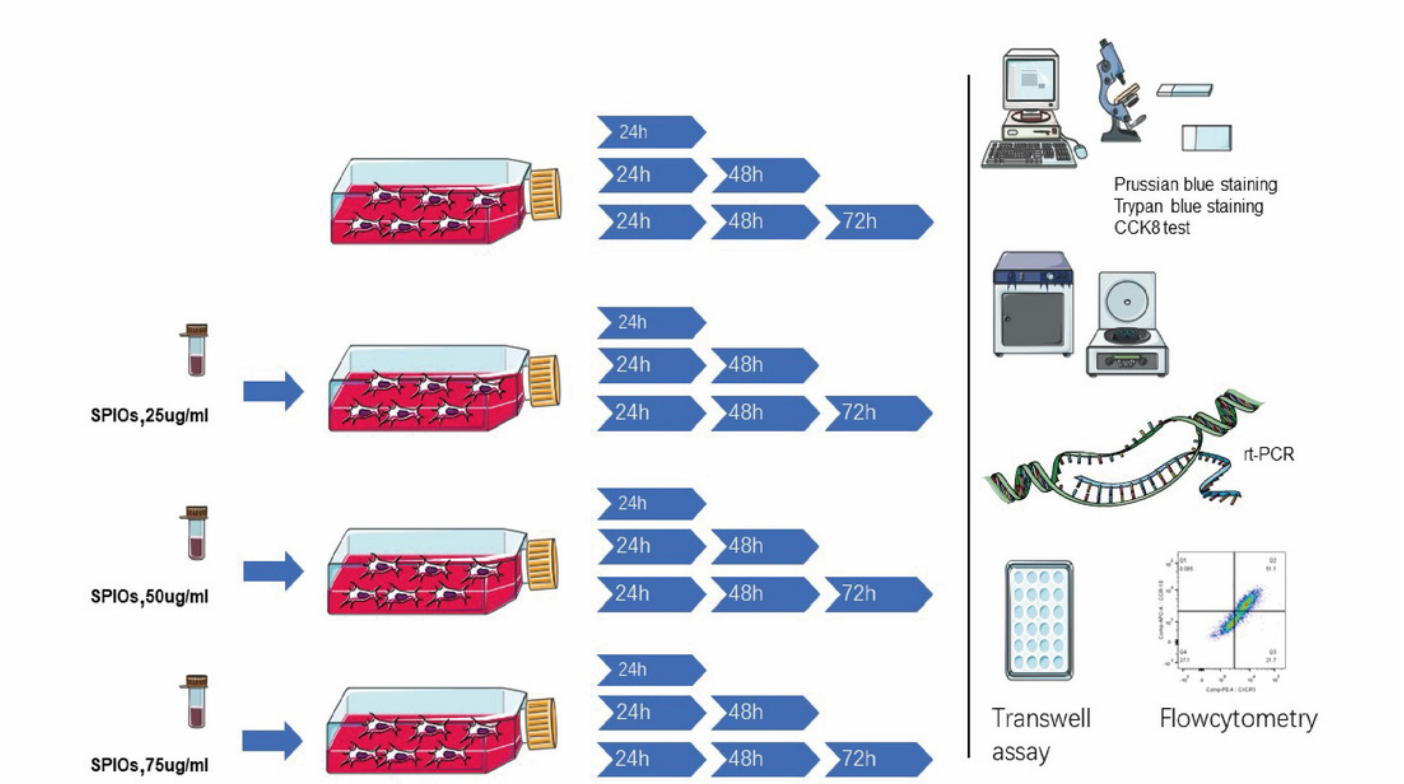

Table 1(on next page)

This is the primier sequences of RT-PCR testing.

The primier sequences of RT-PCR testing.

Table 1. Primer sequences for rever transcription-quantitative polymerase chain reaction analysis.

Gene	Forward(5'-3')	Reverse(5'-3')
CXCR4	TGCCATGGAAATATACACTTCGG	TGCCCACTATGCCAGTCAAG
CCR10	GGTGGCTGTGCTGGGTTTGG	GGAGGTGGGAGATCGGGTAGTTC
CXCR3	GCCAGTCCTCTACAGCCTCCTC	ACAGCCAGGTGGAGCAGGAAG
CXCR5	GAACTCCCCGATATCGCTAGAC	TGGCCAGTTCCTTGTACAGAT
CXCR7	CCGCGAGGTCACTTGGTT	CAGTGTGTGTCGTAGCCTGT
IL-6	GCCCACCAGGAACGAAAGTC	TGGCTGGAAGTCTCTTGCGG
IL-11	CTTCAGACCCTCGTGCAGAT	CAGGAAGCTGCAAAGATCCCA

CXCR4, CXC-chemokine receptor CXCR4; CCR10, CC chemokine receptor CCR10; CXCR3, CXC-chemokine receptor CXCR3; CXCR5, CXC-chemokine receptor CXCR5; CXCR7, CXC-chemokine receptor CXCR7; IL-6, interleukin-6; IL-11, interleukin-11.

Figure 2

The morphology of the BMSCs.

The BMSCs displayed a fibroblast-like appearance throughout culture. Images show BMSCs from passages (A, C) 0 and (B, D) 10 at 50X and 100X magnification as indicated.

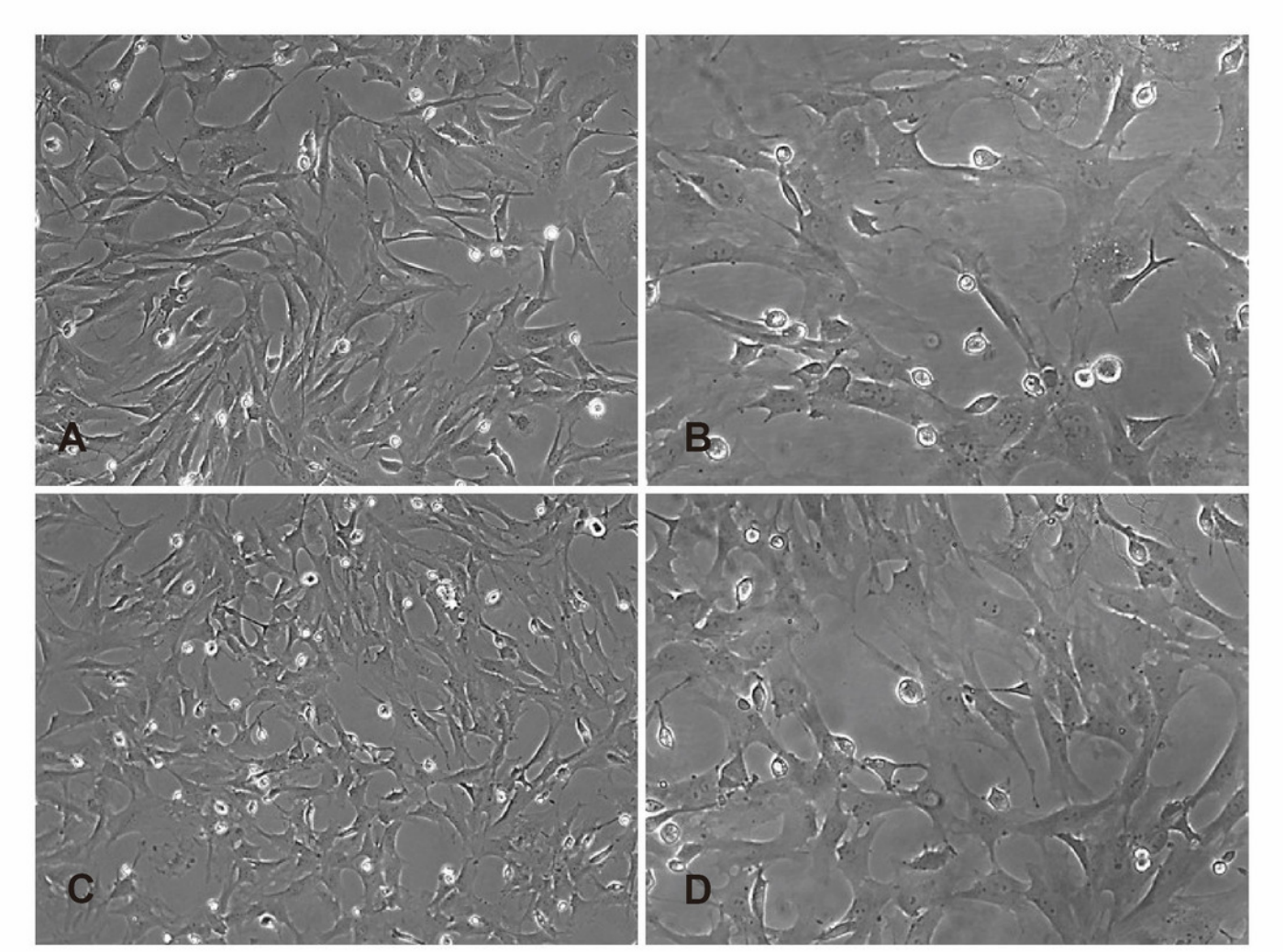

Figure 3

Identification of BMSCs.

Flow cytometry plots showing percentages of (A) CD29+, (B) CD44+, (C) CD34- and (D) CD45- cells.

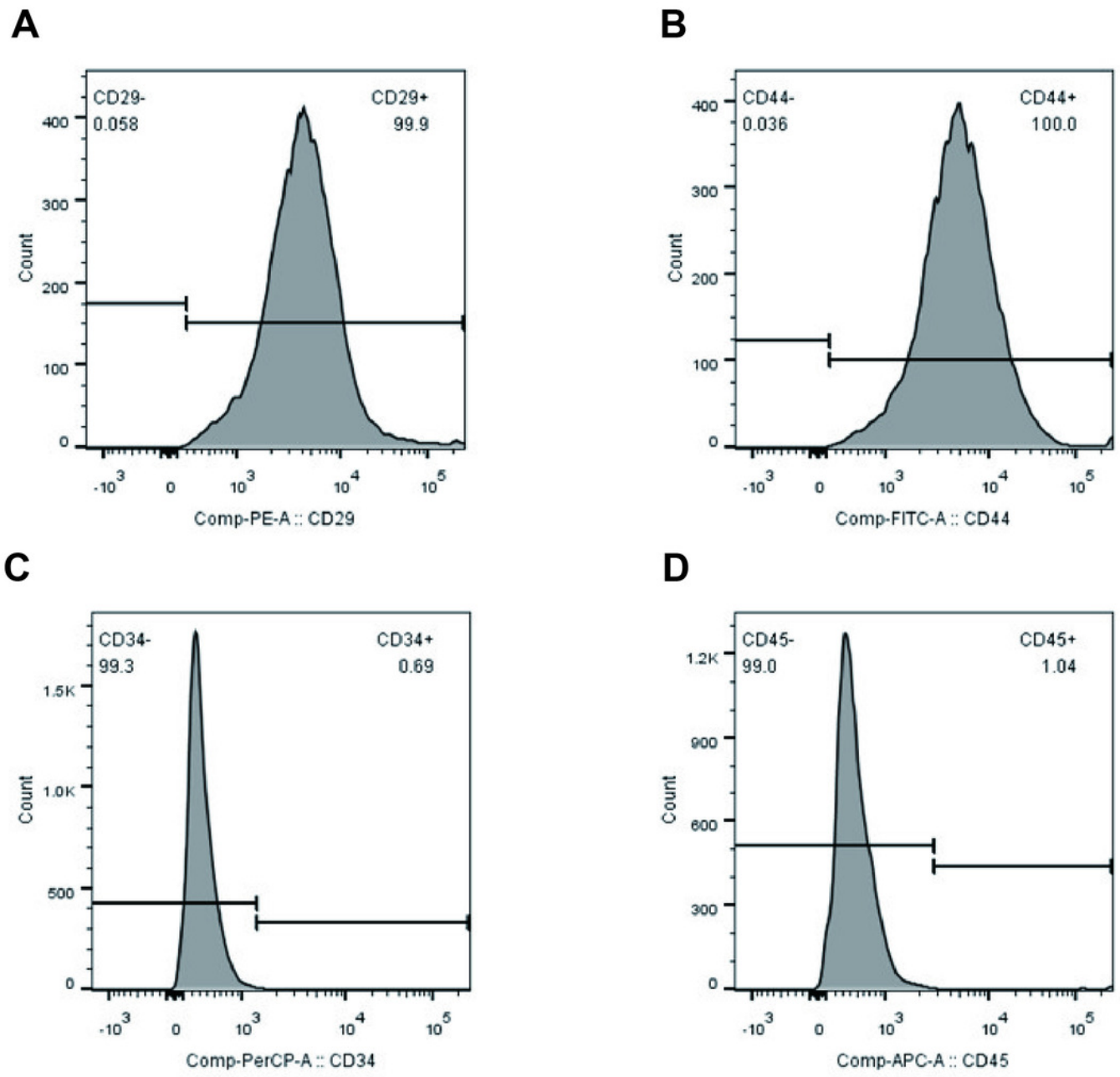

Figure 4

Labelling rate of BMSCs.

(A) Representative images showing Prussian blue-stained iron particles in the BMSCs labeled with different concentrations of SPIOs for varying durations. (B) Labelling rate in the indicated groups. (C) Representative images showing live cells in the indicated groups after trypan blue staining. (D) Viability rates in the indicated groups. (E) Proliferation rates in the indicated groups as measured by CCK-8 test. Data are individual means or the mean ± SD of each group from three separate experiments. *p<0.05, **p<0.01, ***p<0.001

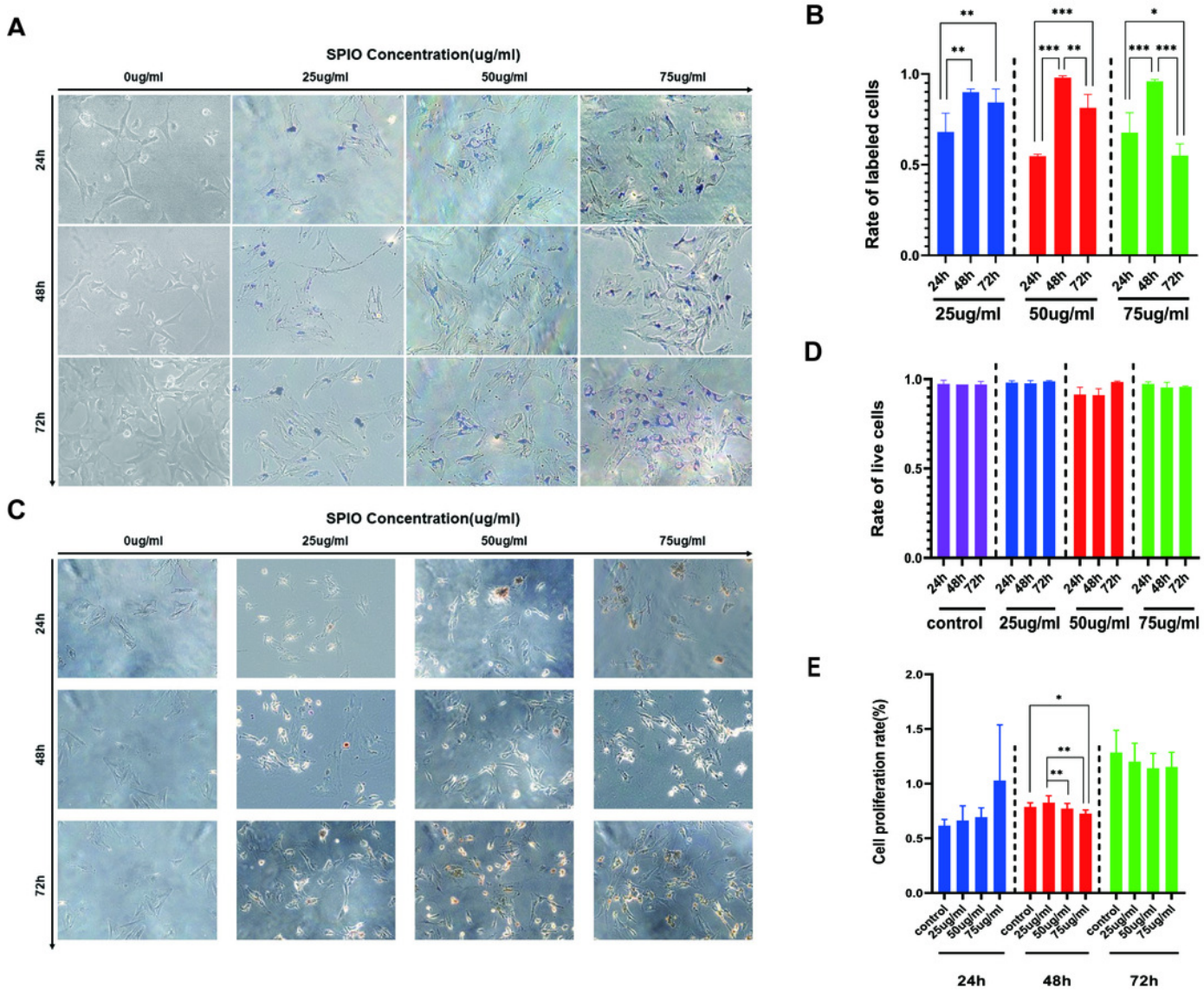
A
SPIO Concentration(ug/ml)
0ug/ml
25ug/ml
50ug/ml
75ug/ml
24h
48h
72h
B
Rate of labeled cells
1.0
0.5
0.0
24h 48h 72h
25ug/ml
50ug/ml
75ug/ml
C
SPIO Concentration(ug/ml)
0ug/ml
25ug/ml
50ug/ml
75ug/ml
24h
48h
72h
D
Rate of live cells
1.0
0.5
0.0
control
25ug/ml
50ug/ml
75ug/ml
E
Cell proliferation rate(%)
2.0
1.5
1.0
0.5
0.0
control
25ug/ml
50ug/ml
75ug/ml
24h
48h
72h

Figure 5

SPIO labelling upregulated chemokine receptor and cytokine genes in BMSCs.

(A, B) The expression level of CCR5, CCR10, CXCR3, CXCR5, IL11, IL 6 and CXCR7 in in the indicated groups. Different colors correspond to the labelling concentrations (25/50/75μg/ml) and culture durations (24/48/72h). Data are individual means or the mean ± SD of each group from three separate experiments. *p<0.05, **p<0.01, ***p<0.001

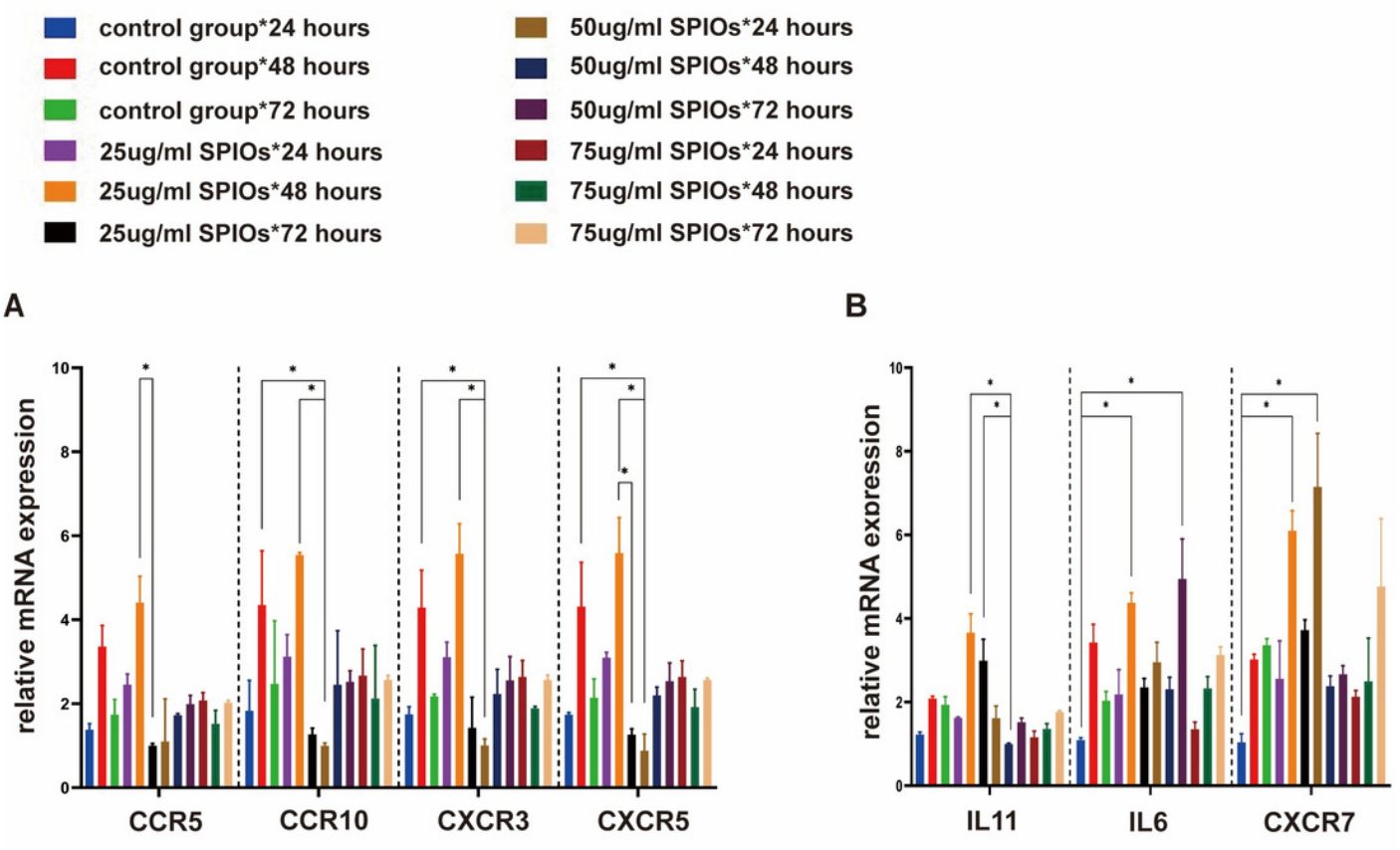

Figure 6

SPIO labelling increased percentage of BMSCs expressing chemokine receptors.

(A) Flow cytometry plots showing percentage of CXCR3/CCR10 control and SPIOs-labeled BMSCs. (D) Flow cytometry plots showing percentage of CXCR7/CXCR4 control and labeled BMSCs. Different colors of the bars on top right correspond to the labelling concentrations of SPIOs. (B) CXCR3 expression in different groups. (C) CCR10 expression in different groups. (E) CXCR7 expression in different groups. (F) CXCR4 expression in different groups. Data are individual means or the mean ± SD of each group from three separate experiments. *p<0.05, **p<0.01, ***p<0.001

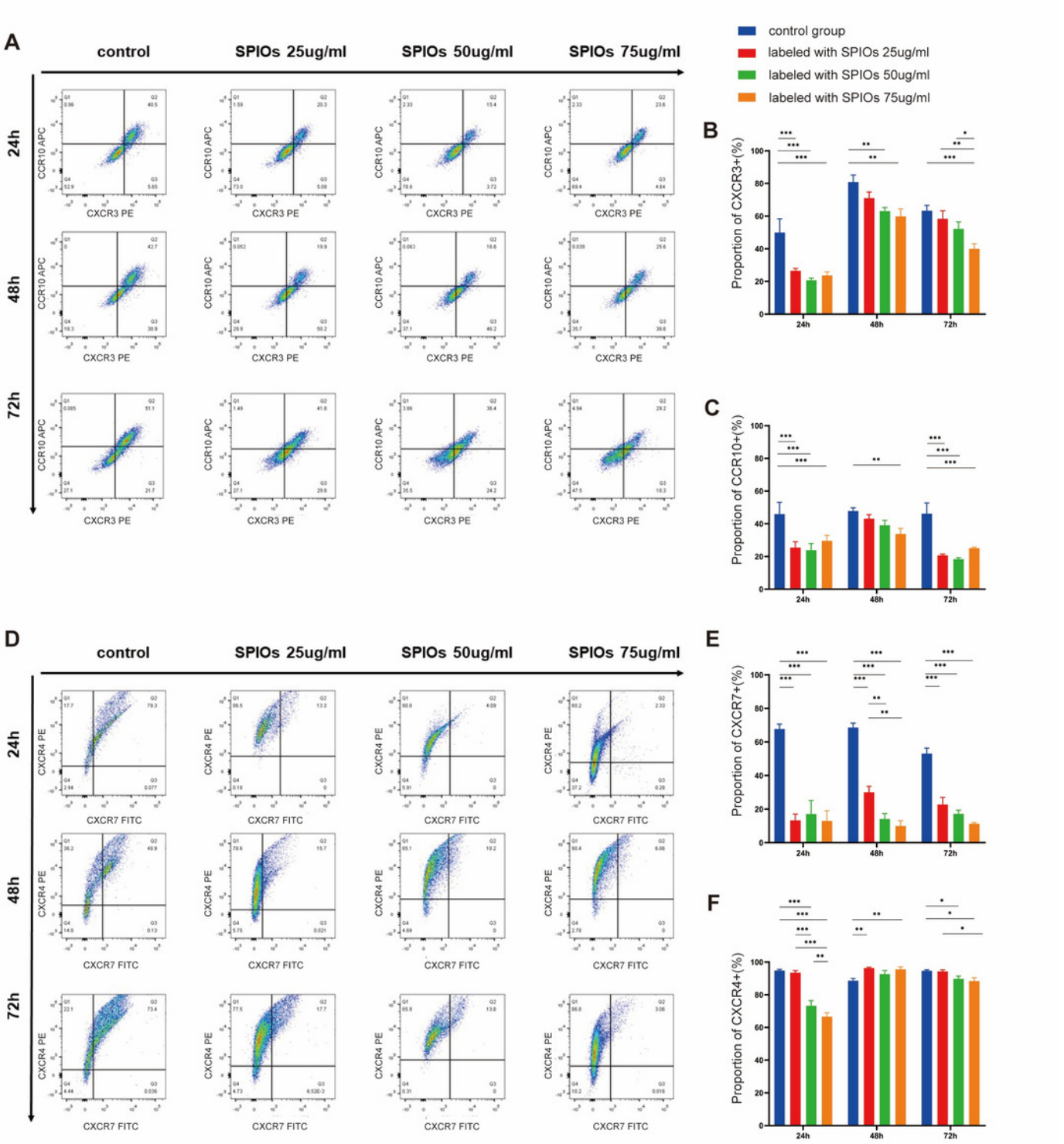
A
control
SPIOs 25ug/ml
SPIOs 50ug/ml
SPIOs 75ug/ml
24h
48h
72h
CCR10 APC
CXCR3 PE
control group
labeled with SPIOs 25ug/ml
labeled with SPIOs 50ug/ml
labeled with SPIOs 75ug/ml
B
Proportion of CXCR3+(%)
C
Proportion of CCR10+(%)
D
CXCR4 PE
CXCR7 FITC
E
Proportion of CXCR7+(%)
F
Proportion of CXCR4+(%)

Figure 7

SPIOs labelling has no influence on the migration of BMSCs.

(A) Representative images of the BMSCs cultured in transwell chambers in the presence or absence of TAK 799, MARAVIORIC and LY294002 with CXCL10, CCL4 or CCL19 in the lower chamber (magnification x10). (B) Migration rates in the indicated groups. Data are expressed as the mean ± SD of each group (n=3). The colors of the bars on top right correspond to the number of migrating cells. *p<0.05, **p<0.01, ***p<0.001