CHAPTER 4

FINDING AND RESULTS

Subject Characteristics

Human bone marrow was obtained from sternum or iliac crest of 5 healthy volunteers of both sexes (age ranging from 24 to 61 years). All donors have no clinical history of any malignancy, metabolic disorder, or infectious disease.

Postnatal tissues (umbilical cord, Wharton's jelly, placenta and amnion) were collected from 5 healthy women (gestational age ranging from 38 to 42 weeks) after normal deliveries at labour room, Siriraj hospital, Mahidol University.

Peripheral blood samples were collected from 5 healthy volunteers (age ranging from 21 to 67 years). All donors have no clinical history of any malignancy, metabolic disorder, or infectious disease.

The characteristics of culture UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs in comparison to BM-MSCs

Mononuclear cells (MNCs) isolated from bone marrow were cultured in DMEM supplemented with 10% FBS at a density of $1x10^5$ cells/cm^2. After plating, MNCs had spherical shape and floated over the culture flask (Fig. 4.2 A). One day after plating, some cells had attached to the flask but still had spherical characteristic. On day 3, non-adherent cells and cell debris were removed. The adherent cells had a heterogeneous characteristic. There were many rounds, monocyte-like cells as well as a number of flattened cells mixed with spindle-shape cells (Fig. 4.2 B). The media were changed every 3 -4 days and the number of rounded monocyte-like cells was steadily diminished over the course of approximately 1 week (Fig. 4.2 C). Cultures were monitored daily and between 7-10 days of culture, small outgrowth colonies of approximately 20-50 cells were observed (Table 1, Fig. 4.2). After that cells were rapidly proliferated (Fig. 4.2 D-F).

The cells isolated from human umbilical cords (UC), Wharton's jelly (WJ), placenta (PL) and amnion (AM) were also cultured in the same condition as the cells isolated from bone marrow. After plating, only a few cells attached to the floor of the culture flasks (Fig. 4.3-4.6 A). The media were changed every 3-4 days and the number of non-adherent cells was steadily diminished over the course of approximately 3 weeks (Fig. 4.3-4.6 C). Cultures were monitored daily and the initial colony formation was observed earlier in UC-culture (7.6±0.88 days) than in BM-culture (8.2±0.75). In contrast, the cells isolated from placenta and amnion took a significantly longer period of time to develop MSCs colonies than bone marrow (18.6±1.05 days, 11.8±1.19 days vs. 8.2±0.75, $P<0.05$). MSCs derived from umbilical cords (UC-MSCs), Wharton's jelly (WJ-MSCs), placenta (PL-MSCs) and amnion (AM-MSCs) have spindle-shaped morphology similar to bone marrow-derived MSCs (BM-MSCs) (Fig. 4.3-4.6 C). Representative photomicrographs of UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs are shown in figure 4.3-4.6, respectively. UC-MSCs reached 90% confluence after approximately 10 days of culture. Further passages of MSCs from these sources were done whenever the cell density reached 90% confluence. We were also able to freeze, thaw, and subsequently passage these MSCs. The resulting UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs can be propagated up to 18-22 passages and can be revived after frozen. At passage 22, the cells show signs of replicative senescence and stop growing. In contrast to those 4 resources, the BM-MSCs can only be propagated up to passage 10 before they reach the stage of replicative senescence.

Table 1

The onset of colony formation

Source of MSCs	Time (days)	*P*-value
Bone marrow	8.20 ± 0.75	-
Umbilical cord	7.60 ± 0.88	0.521
Wharton's jelly	9.80 ± 0.75	0.088
Placenta	18.60 ± 1.05	0.001*
Amnion	11.80 ± 1.19	0.010*

Values are presented as mean ±SEM.

* p<0.05 significantly different compared to control (bone marrow)

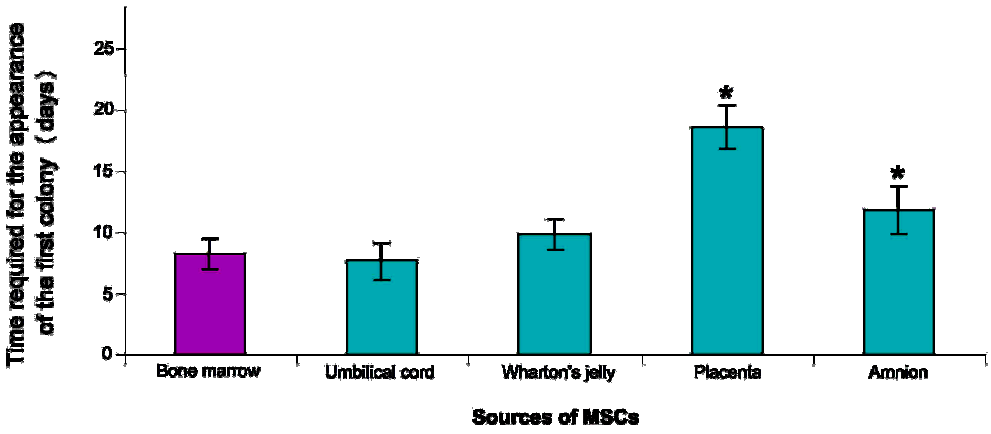

Figure 4.1 The onset of colony formation. Data are presented as mean±SEM. *P<0.05: significantly different compared to control (BM-MSCs).

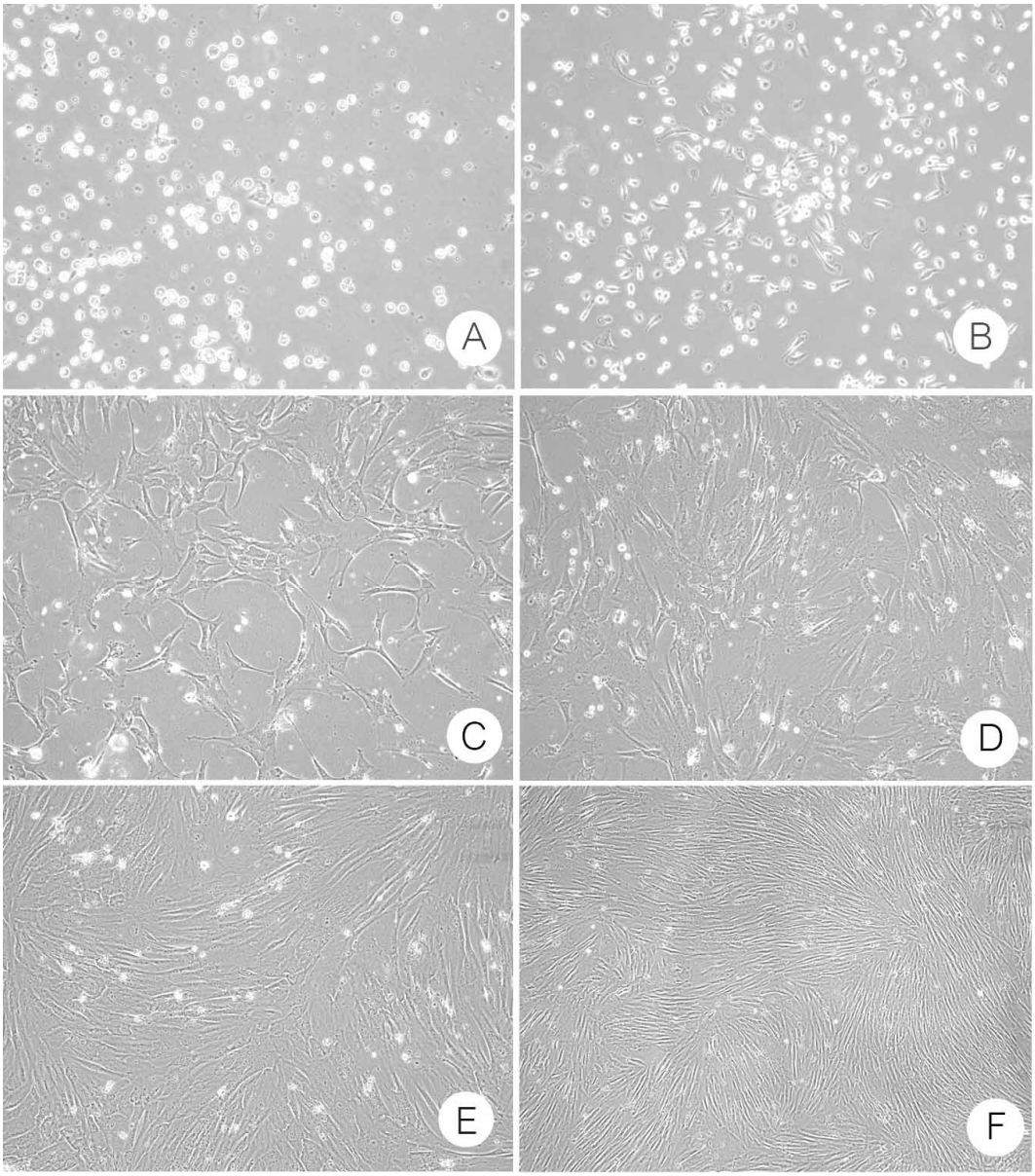

Figure 4.2 Morphology of mesenchymal stem cells derived from bone marrow (BM-MSCs). A: Spherical-shaped cells at day 0 after the initial seeding; B: Adherent cells at day 3; C: Spindle-shaped cells at day 7; D: BM-MSCs was reached sub-confluence at day14; E: BM-MSCs was reached 100% confluence at day16; F: BM-MSCs at passage 8 (100% confluence).

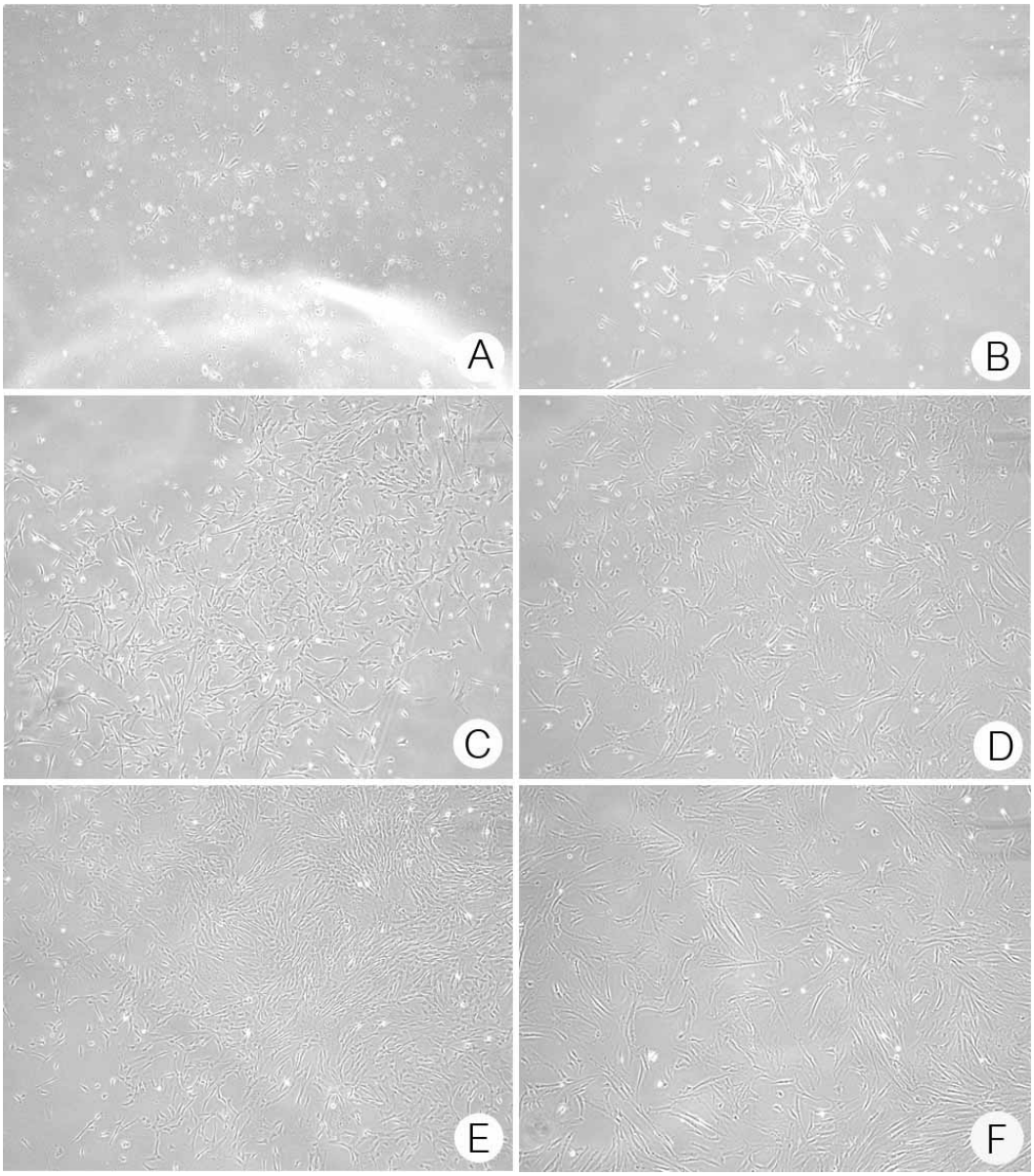

Figure 4.3 Morphology of mesenchymal stem cells derived from umbilical cord (UC-MSCs). A: Spherical-shaped cells after the initial seeding; B: Adherent cells at day 3; C: Spindle-shaped cells at day 7; D: UC-MSCs was reached sub-confluence at day14; E: UC-MSCs was reached 100% confluence at day15; F: UC-MSCs at passage 21 (100% confluence).

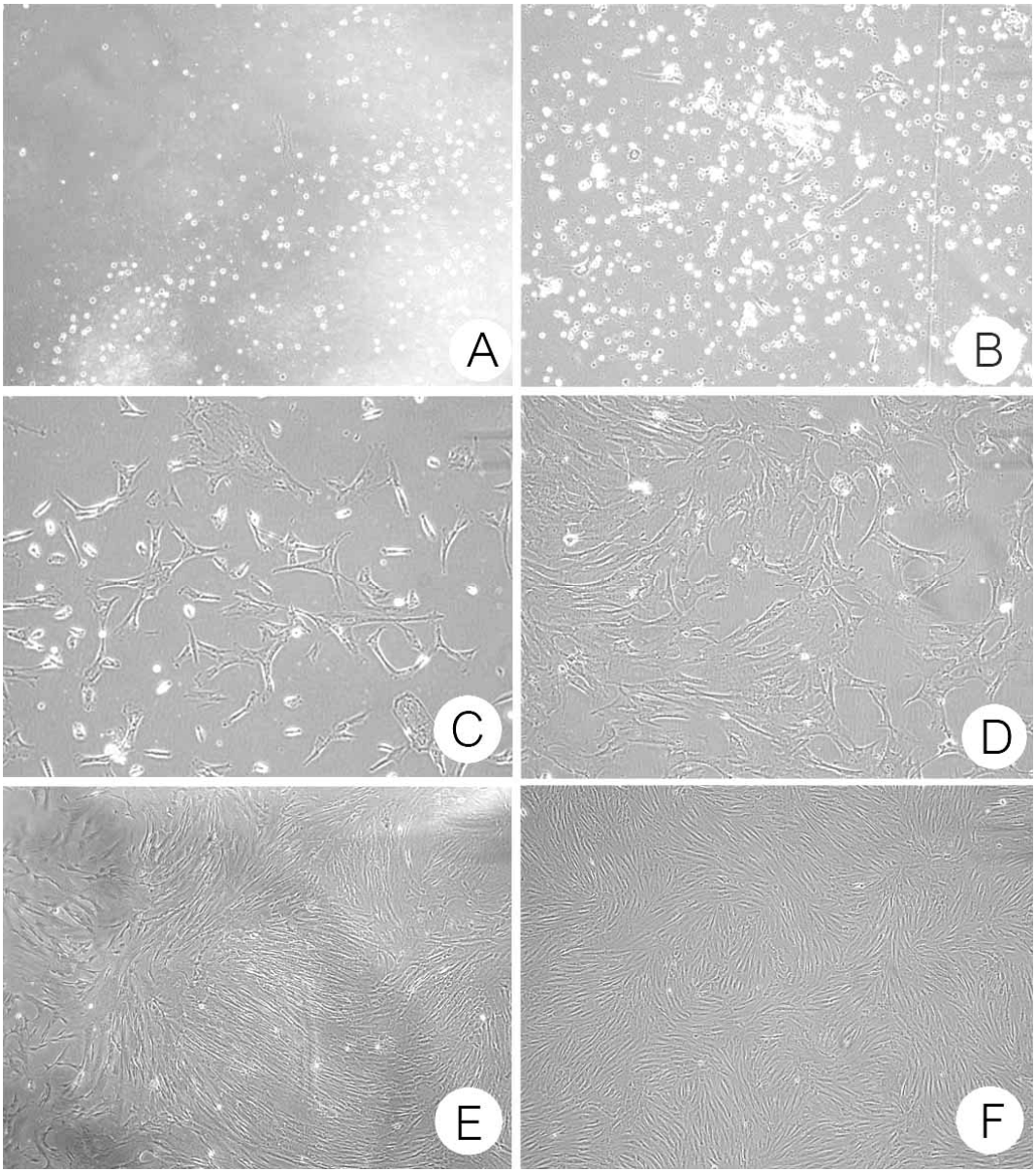

Figure 4.4 Morphology of mesenchymal stem cells derived from Wharton's jelly (WJ-MSCs). A: Spherical-shaped cells after the initial seeding; B: Adherent cells at day 3; C: Spindle-shaped cells at day 7; D: WJ-MSCs was reached sub-confluence at day14; E: WJ-MSCs was reached 100% confluence at day20; F: WJ-MSCs at passage 12 (100% confluence).

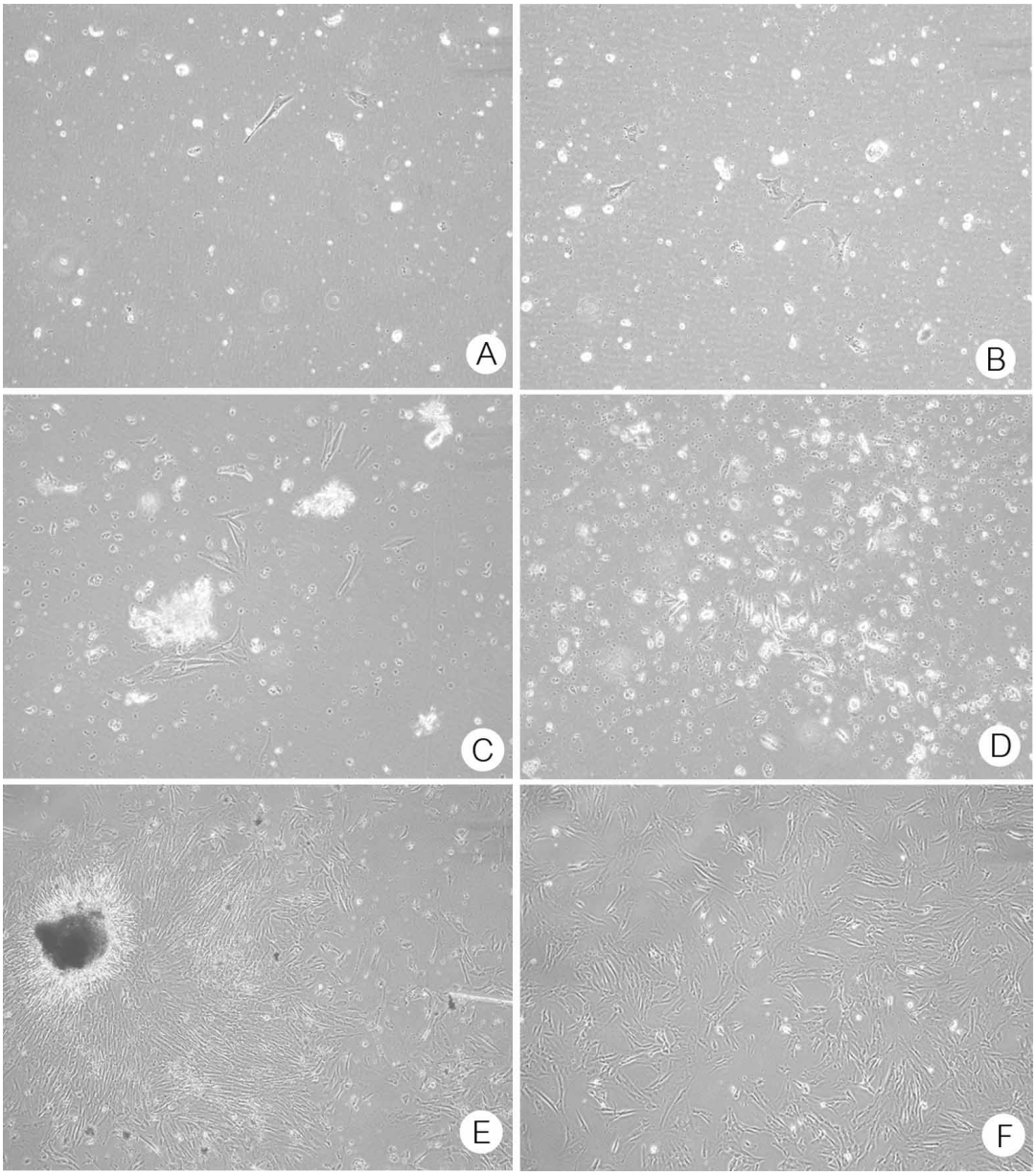

Figure 4.5 Morphology of mesenchymal stem cells derived from placenta (PL-MSCs). A: Spherical-shaped cells after the initial seeding; B: Adherent cells at day 3; C: Spindle-shaped cells at day 7; D: Spindle-shaped cells at day 14; E: PL-MSCs was reached sub-confluence at day 25; F: PL-MSCs at passage 10 (100% confluence).

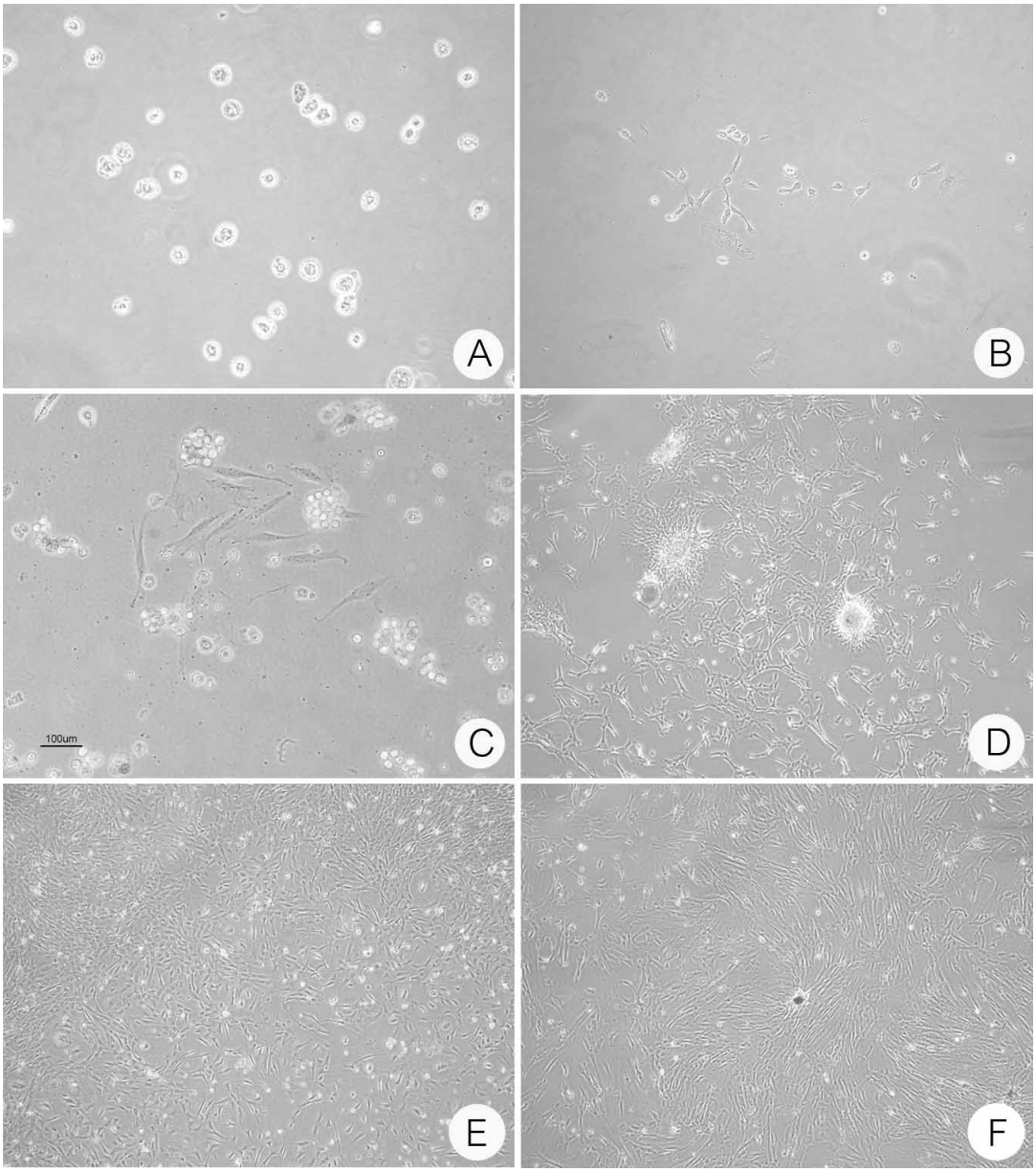

Figure 4.6 Morphology of mesenchymal stem cells derived from amnion (AM-MSCs). A: Spherical-shaped cells after the initial seeding; B: Adherent cells at day 3; C: Spindle-shaped cells at day 7; D: Spindle-shaped cells at day 14; E: AM-MSCs was reached sub-confluence at day 27; F: AM-MSCs at passage 9 (100% confluence).

The proliferation characteristics of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs in comparison to BM-MSCs

The growth characteristics of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs and BM-MSCs were determined during 8 days. The results showed that UC- MSCs and WJ-MSCs have a higher proliferative capacity than BM-MSCs, PL-MSCs and AM-MSCs. The proliferative capacity of UC- MSCs during early period (passage 2 and 3) was similar to that of WJ-MSCs (Fig. 4.7 A, B). There was no significant different in cell number and growth kinetics during the 4 days of culture. Between day 4 and 6, the number of UC-MSCs was significantly higher compared to those of WJ-MSCs ($P<0.05$). After 6 day of culture, the cell density reach 100% confluence and their growth stopped. The proliferative capacity of BM-MSCs, during the first 4 days was significantly lower than those of UC- MSCs and WJ-MSCs ($P<0.05$) (Fig. 4.7 A, B). In contrast, the proliferative capacity of PL-MSCs and AM-MSCs were significantly lower than that of BM-MSCs in all passages examined ($P<0.05$) (Fig. 4.7 A-D).

Table 2

Proliferation potential of MSCs at passage 2

Source	Number of cells at day 2 ($x10^4$ cells)	Number of cells at day 4 ($x10^4$ cells)	Number of cells at day 6 ($x10^4$ cells)	Number of cells at day 8 ($x10^4$ cells)
Bone marrow	1.37 ± 0.74	1.99 ± 0.75	5.27 ± 0.84	6.62 ± 0.87
Umbilical cord	1.21 ± 1.28	3.84 ± 1.13	21.21 ± 1.53	22.66 ± 1.48
Wharton's jelly	1.01 ± 0.98	3.25 ± 0.92	11.86 ± 1.15	19.86 ± 1.02
Amnion	1.10 ± 0.55	1.26 ± 0.47	3.033 ± 0.49	5.23 ± 0.53
Placenta	1.01 ± 0.77	1.18 ± 0.81	2.98 ± 0.76	4.98 ± 0.75

Values are presented as mean ±SEM.

Table 3

Proliferation potential of MSCs at passage 3

Source	Number of cells at day 2 ($x10^4$ cells)	Number of cells at day 4 ($x10^4$ cells)	Number of cells at day 6 ($x10^4$ cells)	Number of cells at day 8 ($x10^4$ cells)
Bone marrow	1.54 ± 0.64	4.15 ± 0.81	9.54 ± 0.76	11.56 ± 0.88
Umbilical cord	3.45 ± 1.77	5.89 ± 1.78	24.51 ± 1.88	26.46 ± 1.94
Wharton's jelly	1.73 ± 1.12	7.52 ± 1.24	22.28 ± 0.98	25.26 ± 1.08
Amnion	2.06 ± 0.51	4.18 ± 0.51	8.93 ± 0.52	11.25 ± 0.52
Placenta	2.00 ± 0.81	3.66 ± 0.79	6.16 ± 0.80	6.58 ± 0.83

Values are presented as mean ±SEM.

Table 4

Proliferation potential of MSCs at passage 4

Source	Number of cells at day 2 ($x10^4$ cells)	Number of cells at day 4 ($x10^4$ cells)	Number of cells at day 6 ($x10^4$ cells)	Number of cells at day 8 ($x10^4$ cells)
Bone marrow	3.19 ± 0.84	12.02 ± 0.92	13.95 ± 0.88	15.11 ± 0.74
Umbilical cord	4.73 ± 1.98	12.98 ± 1.87	21.63 ± 1.95	23.64 ± 2.15
Wharton's jelly	2.60 ± 1.35	10.30 ± 1.54	17.46 ± 1.35	19.10 ± 1.48
Amnion	1.92 ± 0.49	6.41 ± 0.53	10.95 ± 0.51	12.56 ± 0.53
Placenta	1.94 ± 0.75	5.41 ± 0.77	10.96 ± 0.80	13.26 ± 0.79

Values are presented as mean ±SEM.

Table 5

Proliferation potential of MSCs at passage 5

Source	Number of cells at day 2 ($x10^4$ cells)	Number of cells at day 4 ($x10^4$ cells)	Number of cells at day 6 ($x10^4$ cells)	Number of cells at day 8 ($x10^4$ cells)
Bone marrow	4.54 ± 0.87	13.32 ± 0.71	14.82 ± 0.82	14.82 ± 0.71
Umbilical cord	4.10 ± 1.67	11.53 ± 1.54	15.80 ± 1.65	16.91 ± 1.74
Wharton's jelly	3.30 ± 0.99	4.50 ± 1.15	10.66 ± 1.23	12.58 ± 1.18
Amnion	1.23 ± 0.52	2.54 ± 0.49	4.53 ± 0.51	5.98 ± 5.23
Placenta	1.16 ± 0.75	2.41 ± 0.81	4.16 ± 0.79	6.33 ± 0.80

Values are presented as mean ±SEM.

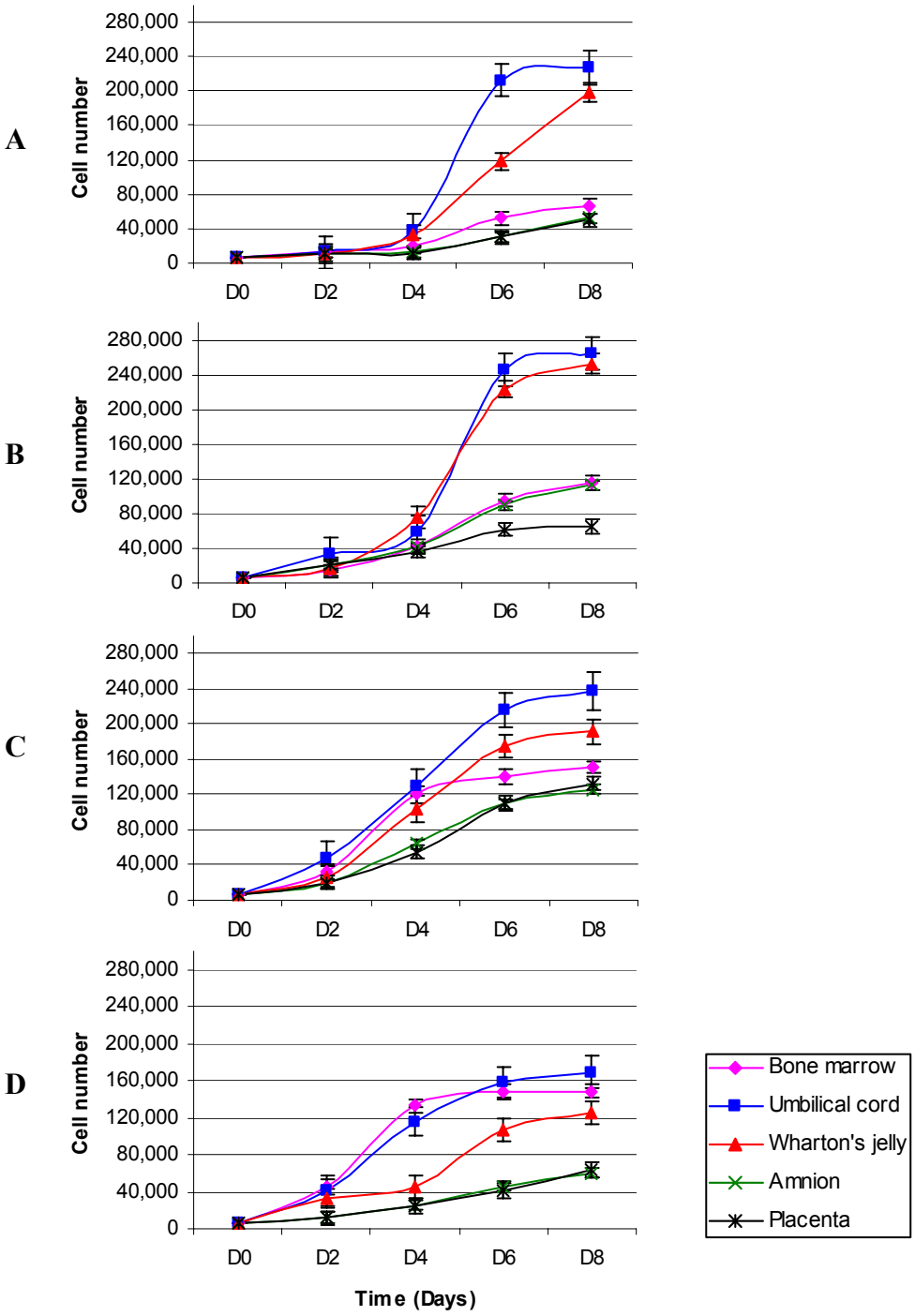

Figure 4.7 Growth curves of MSCs derived from bone marrow, umbilical cord, Wharton's jelly, placenta and amnion. At t=0, $6x10^3$ MSCs were seeded in culture plates. Triplicate cultures were harvested at 2, 4, 6, 8 days, and adherent cells were counted. Results are expressed as mean ± standard error of the mean. A: Growth curve of MSCs at passage 2, B: Growth curve of MSCs at passage 3, C: Growth curve of MSCs at passage 4, D: Growth curve of MSCs at passage 5.

Cell surface marker characteristics of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs in comparison to BM-MSCs

Immunophenotype of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs and BM-MSCs (passage 2 to passage 5) were determined by flow cytometry with use of phycoerythrin(PE)-conjugated or fluorescein isothiocyanate (FITC)-conjugated antibodies against CD34, CD45, CD73, CD90 and CD 105.

UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs exhibit the similar immunophenotype as those of BM-MSCs which expressed high levels of MSC markers (CD73, CD90, and CD105) but did not expressed hematopoietic markers (CD34, CD45) (Fig 4.8-4.12). Figure 4.13 summarized the expression profiles of the MSC and hematopoietic cell surface markers of BM-MSCs, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs as determined by flow cytometry.

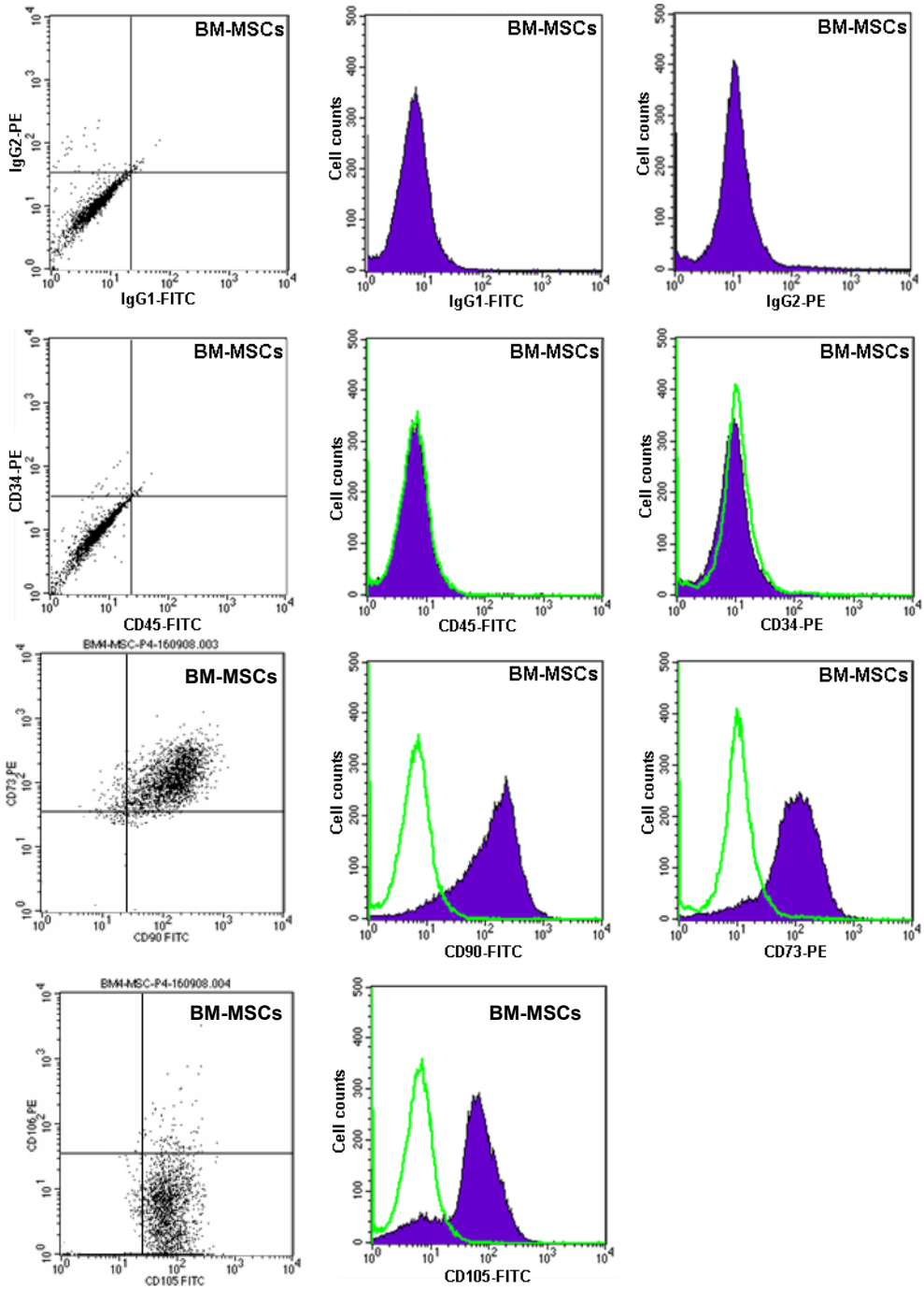

Figure 4.8 Flow cytometric analysis of surface-marker expression on bone marrow derived MSCs. The cells were cultured in DMEM supplemented with 10% FBS. At passage 2, the cells were analyzed by flow cytometry. The green line shows the profile of negative control. The data shown are representative of those obtained in three different experiments.

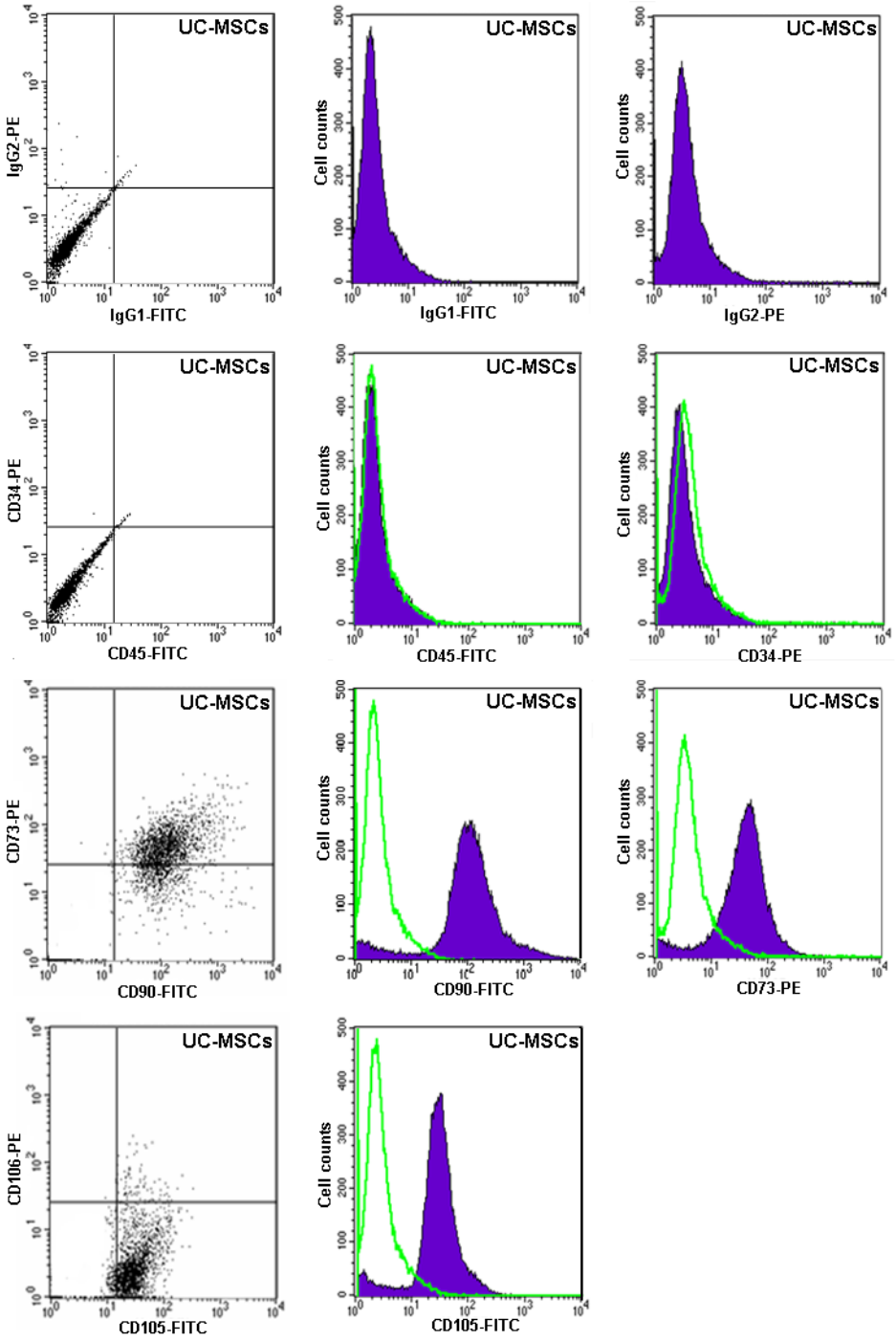

Figure 4.9 Flow cytometric analysis of surface-marker expression on umbilical cord derived MSCs. The cells were cultured in DMEM supplemented with 10% FBS. At passage 2, the cells were analyzed by flow cytometry. The green line shows the profile of negative control. The data shown are representative of those obtained in three different experiments.

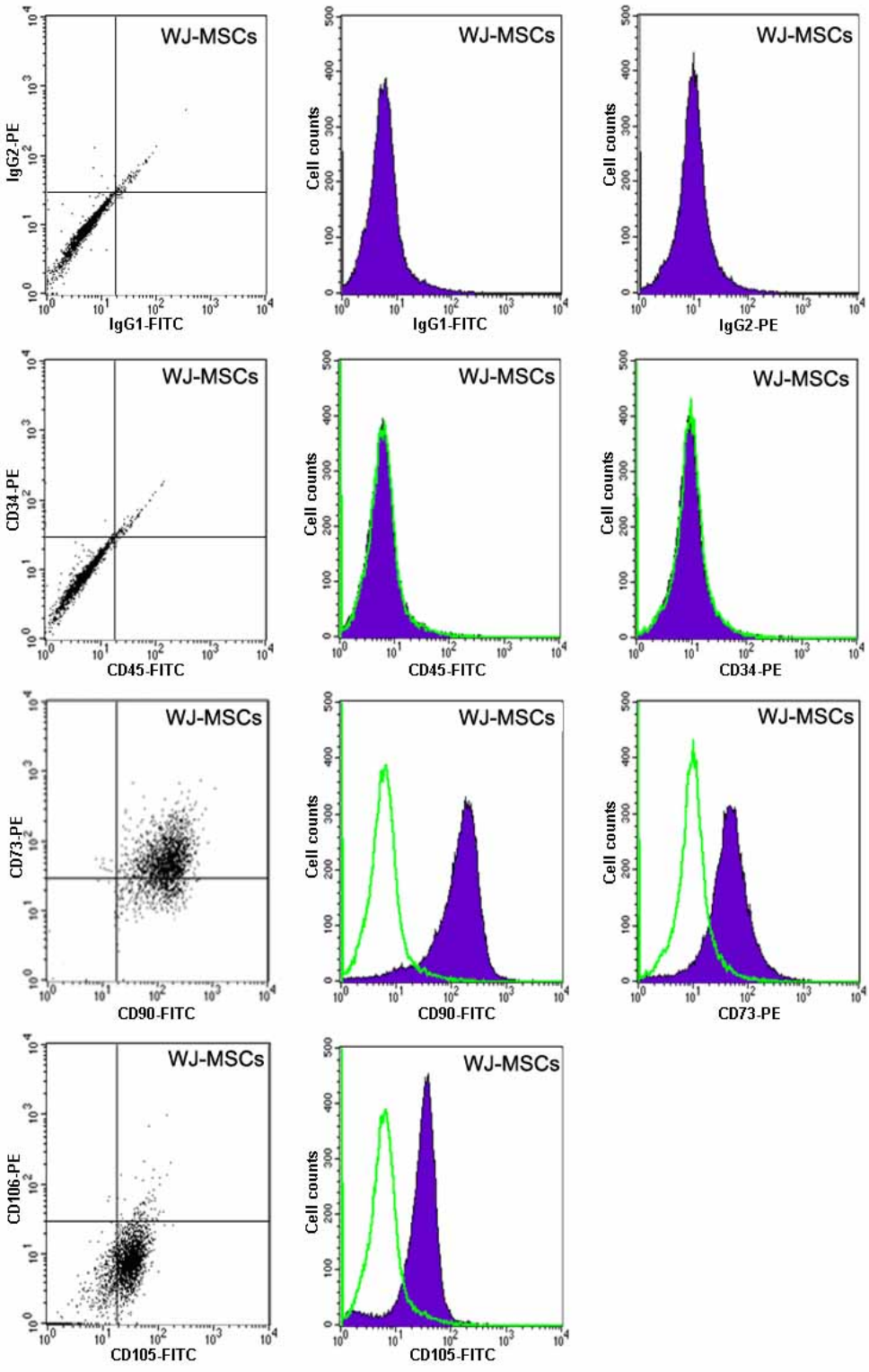

Figure 4.10 Flow cytometric analysis of surface-marker expression on Wharton's jelly derived MSCs. The cells were cultured in DMEM supplemented with 10% FBS. At passage 2, the cells were analyzed by flow cytometry. The green line shows the profile of negative control. The data shown are representative of those obtained in three different experiments.

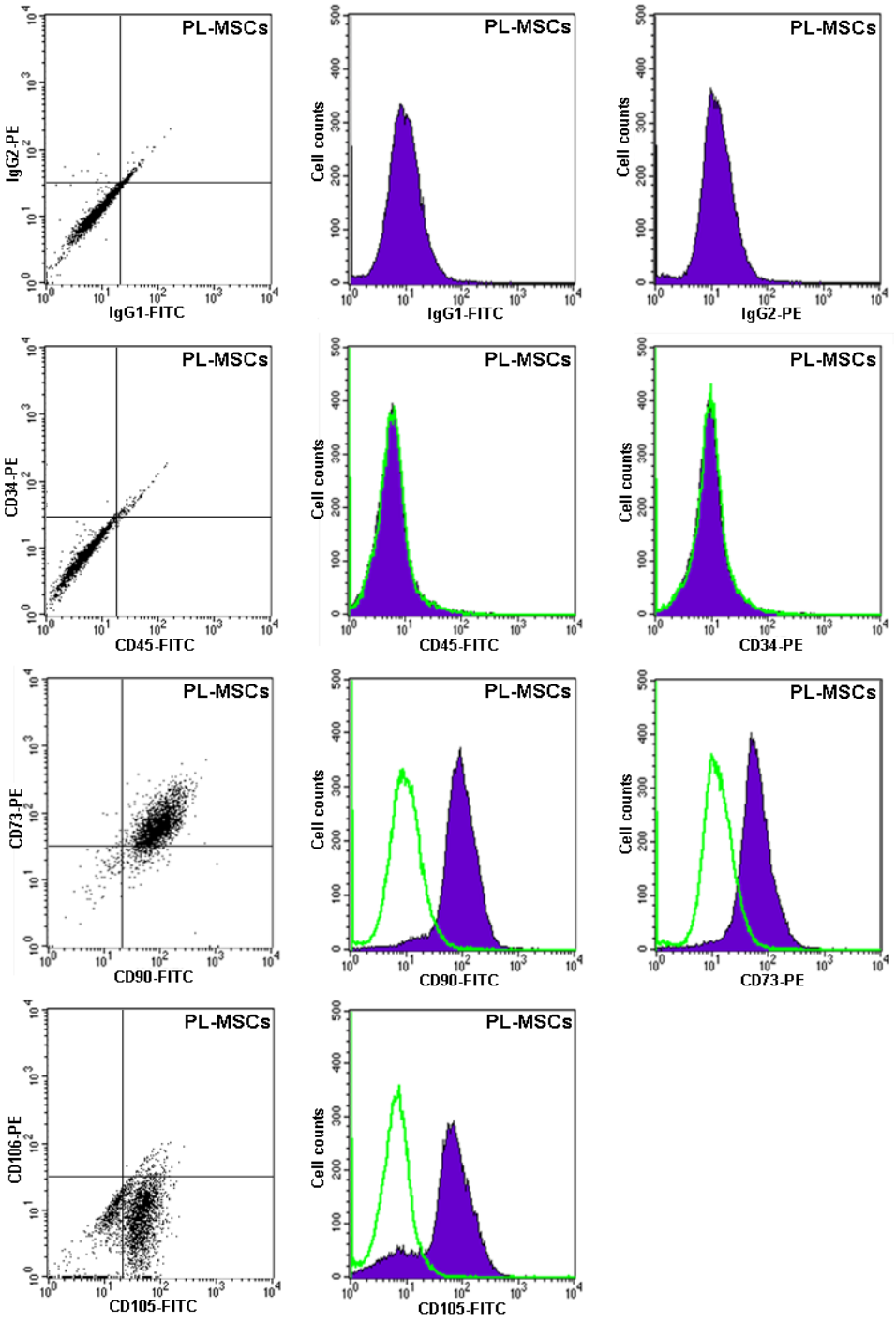

Figure 4.11 Flow cytometric analysis of surface-marker expression on placenta derived MSCs. The cells were cultured in DMEM supplemented with 10% FBS. At passage 2, the cells were analyzed by flow cytometry. The green line shows the profile of negative control. The data shown are representative of those obtained in three different experiments.

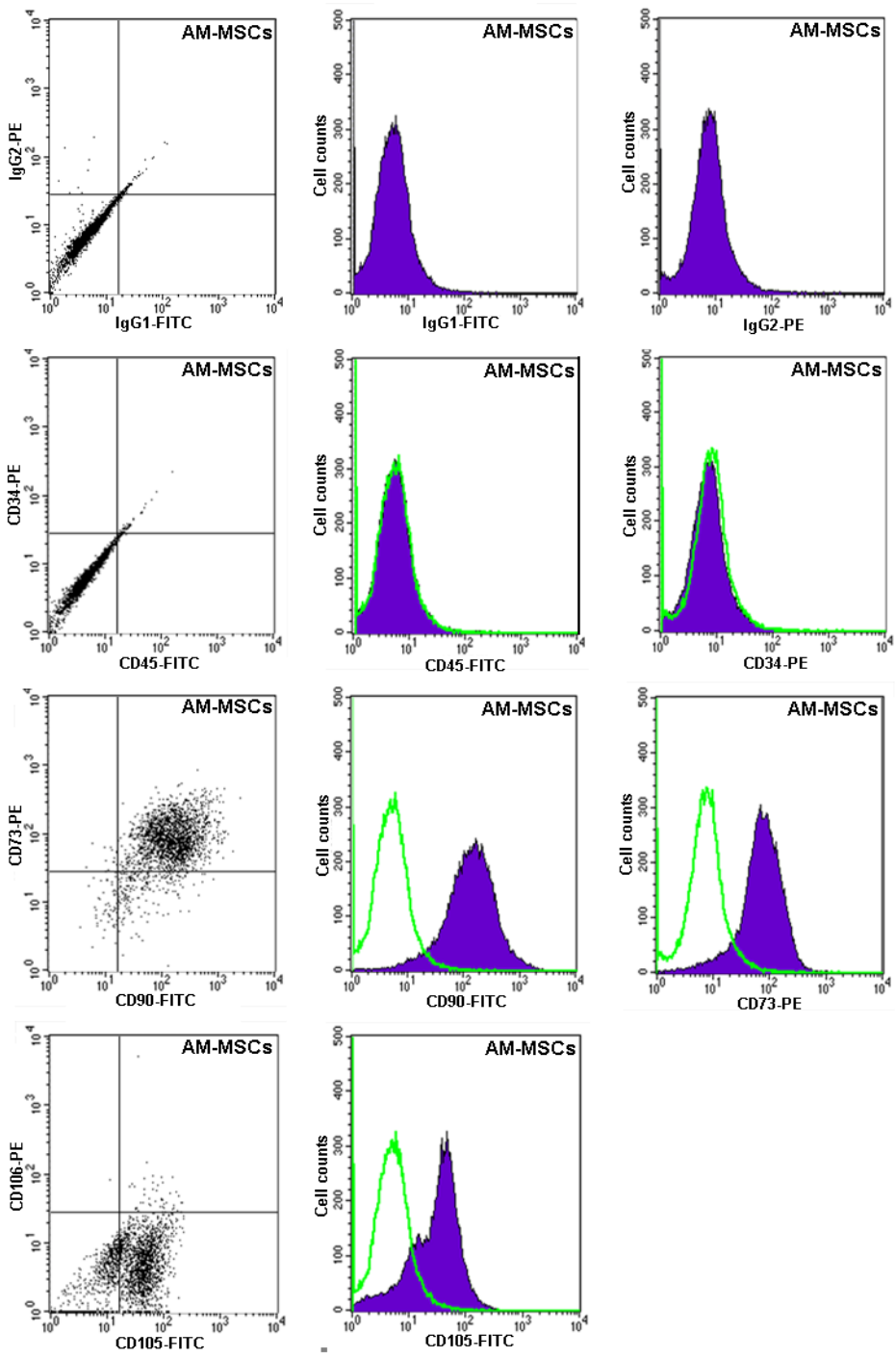

Figure 4.12 Flow cytometric analysis of surface-marker expression on amnion derived MSCs. The cells were cultured in DMEM supplemented with 10% FBS. At passage 2, the cells were analyzed by flow cytometry. The green line shows the profile of negative control. The data shown are representative of those obtained in three different experiments.

Table 6

The expression of surface-marker on MSCs at passage 2

Surface marker	Number of surface-marker expression (%)				
	BM-MSCs	**UC-MSCs**	**WJ-MSCs**	**PL-MSCs**	**AM-MSCs**
CD34	5.07 ± 0.05	3.70 ± 1.40	3.40 ± 1.63	4.48 ± 2.51	3.02 ± 0.52
CD45	5.20 ± 0.92	4.83 ± 1.89	5.09 ± 1,70	4.90 ± 2.81	1.86 ± 1.06
CD73	91.55 ± 2.84	76.93 ± 7.47	78.89 ± 5.93	74.84 ± 4.61	97.03 ± 1.36
CD90	90.09 ± 3.83	97.97 ± 2.09	93.31 ± 2.71	79.52 ± 2.43	98.12 ± 1.06
CD105	88.66 ± 2.52	88.26 ± 4.51	74.71 ± 4.28	77.75 ± 4.01	85.12 ± 4.23

Values are presented as mean ±SEM.

Table 7

The expression of surface-marker on MSCs at passage at passage 3

	Number of surface-marker expression (%)				
Surface marker	**BM-MSCs**	**UC-MSCs**	**WJ-MSCs**	**PL-MSCs**	**AM-MSCs**
CD34	5.91 ± 2.73	5.4 ± 3.05	5.79 ± 0.24	4.91± 0.56	4.22 ± 1.36
CD45	5.9 ± 2.43	5.6 ± 1.30	5.75 ± 3.32	5.11 ± 1.86	4.32 ± 0.90
CD73	81.5 ± 2.60	87.56 ± 5.31	85.86 ± 2.91	77.86 ± 3.20	89.78 ± 1.18
CD90	83.63 ± 1.37	93.52 ± 5.45	96.58 ± 4.36	79.41 ± 5.83	96.87 ± 0.31
CD105	65.8 ± 4.21	82.97 ± 2.90	76.43 ± 5.75	75.57 ± 3.46	77.13 ± 8.77

Values are presented as mean ±SEM.

Table 8

The expression of surface-marker on MSCs at passage at passage 4

Surface marker	Number of surface-marker expression (%)				
	BM-MSCs	**UC-MSCs**	**WJ-MSCs**	**PL-MSCs**	**AM-MSCs**
CD34	5.21 ± 1.89	3.84 ± 2.15	3.37 ± 2.01	5.41 ± 2.10	5.87 ± 1.24
CD45	1.42 ± 0.93	2.91 ± 2.05	3.18 ± 1.74	5.75 ± 1.81	5.86 ± 1.04
CD73	83.40 ± 4.08	72.11 ± 3.17	74.93 ± 6.24	96.87 ± 1.54	97.1 ± 2.38
CD90	87.79 ± 2.94	92.67 ± 3.15	95.00 ± 2.97	95.73 ± 2.33	94.67 ± 2.99
CD105	79.71 ± 2.80	82.88 ± 4.21	81.91 ± 2.50	80.4 ± 2.32	85.68 ± 1.84

Values are presented as mean ±SEM.

Table 9

The expression of surface-marker on MSCs at passage at passage 5

	Number of surface-marker expression (%)				
Surface marker	**BM-MSCs**	**UC-MSCs**	**WJ-MSCs**	**PL-MSCs**	**AM-MSCs**
CD34	4.6 ± 0.99	5.18 ± 2.18	1.35 ± 0.76	5.52 ± 1.42	5.6 ± 0.90
CD45	4.54 ± 1.10	5.77 ± 2.36	2.09 ± 0.56	5.07 ± 2.46	5.12 ± 1.06
CD73	61.07 ± 5.16	87.7 ± 5.58	78.15 ± 8.08	71.78 ± 3.52	82.01 ± 2.21
CD90	80.42 ± 2.39	95.16 ± 5.45	94.9 ± 3.09	79.04 ± 1.46	86.82 ± 2.85
CD105	64.01 ± 4.99	84.91 ± 5.79	68.64 ± 3.71	73.5 ± 5.57	80.06 ± 2.10

Values are presented as mean ±SEM.

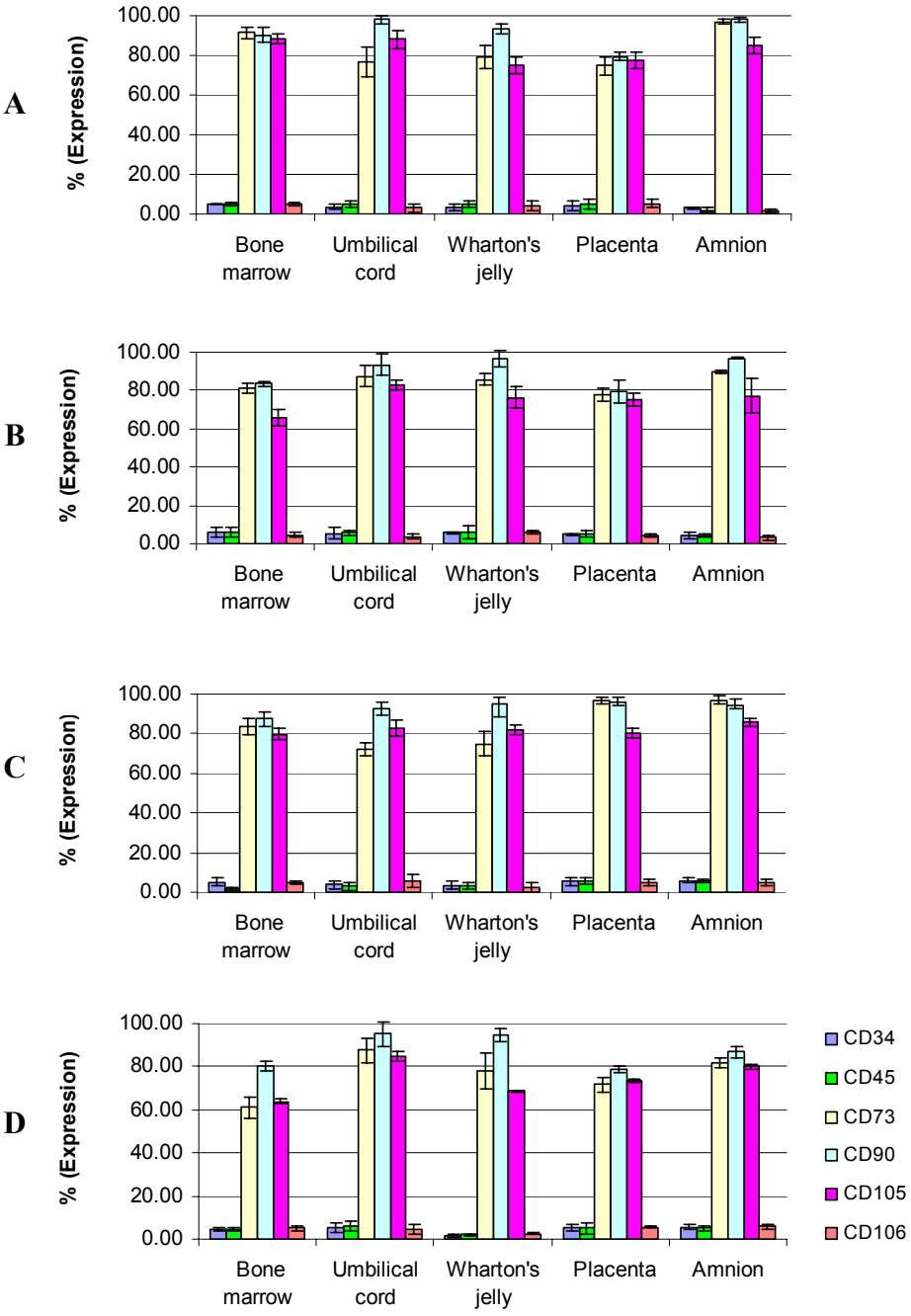

Figure 4.13 The percentages of BM-MSCs, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs that were tested for each cell surface antigen expression using flow cytometry. A: MSCs at passage 2 B: MSCs at passage 3 C: MSCs at passage 4 D: MSCs at passage 5. The data are presented as mean values ± standard error of means. The data shown are representative of those obtained in three different experiments.

Osteogenic differentiation potential of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs in comparison to BM-MSCs.

The osteogenic differentiation potential of UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs were compared to BM-MSCs. MSCs from passage 3 were cultured under condition that is favorable for an osteogenic differentiation. After 10 days of induction, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs had the appearance of refringent crystals in the cells similar to BM-MSCs. By the end of culture, most culture UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs expressed alkaline phosphatase-positive similar to BM-MSCs (Fig. 4.14 B, D, F, H, J). The untreated control cultures growing in regular medium without any osteogenic differentiation stimuli did not expressed alkaline phosphatase even after 3 weeks of culture (Fig. 4.14 A, C, E, G, I).

Adipogenic differentiation potential of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs in comparison to BM-MSCs.

The adipogenic differentiation potential of UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs were compared to BM-MSCs. MSCs from passage 3 were cultured under condition that is favorable for an adipogenic differentiation. After 3 weeks of induction, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs became large cells containing numerous lipid droplets in their cytoplasm similar to that of differentiating BM-MSCs. These lipid droplets were positive for oil red-O staining (Fig. 4.15 B, D, F, H, J,) while the MSCs from untreated control cultures did not have any lipid droplets in their cytoplasm (Fig. 4.15 A, C, E, F, G, I). It is worth noting that PL-MSCs and AM-MSCs need a longer period of time to differentiate into adipogenic lineage in comparison to BM-MSCs, UC-MSCs and WJ-MSCs.

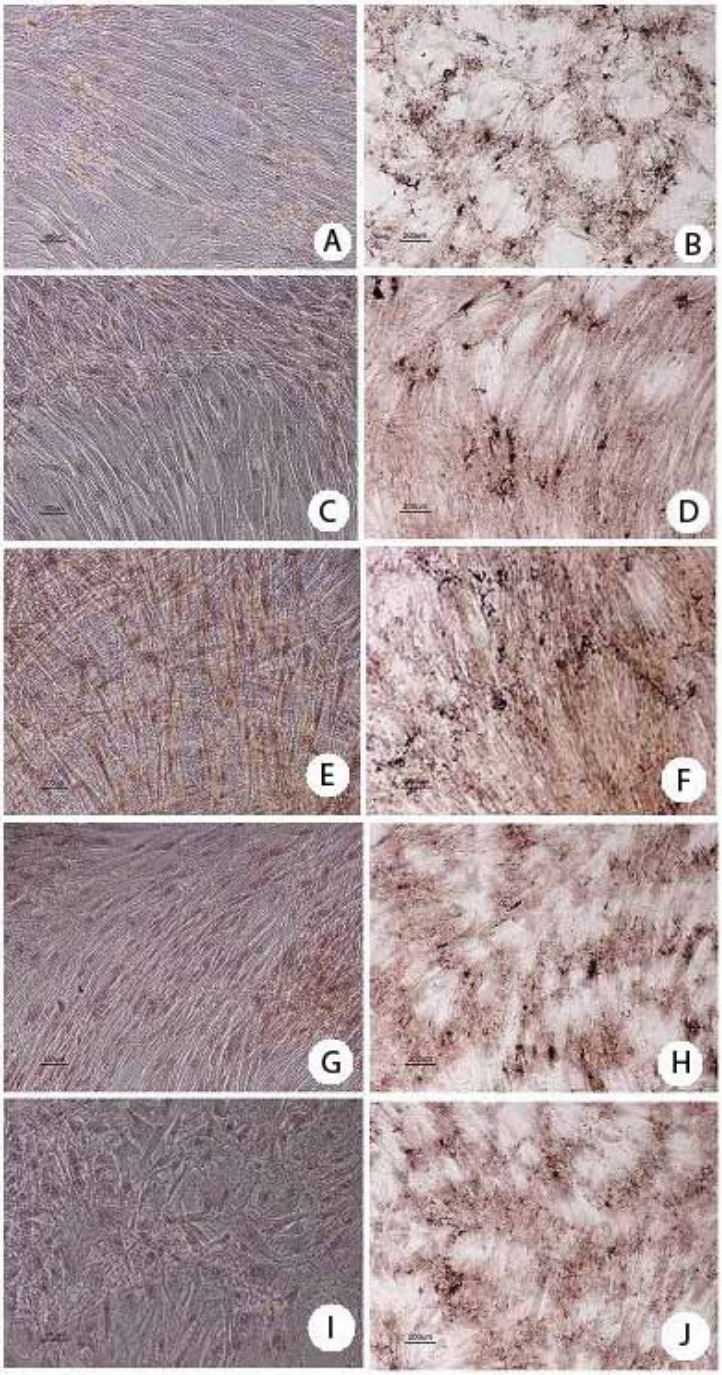

Figure 4.14 Representative photomicrographs of osteogenic differentiation of BM-MSCs (A, B), UC-MSCs (C, D), WJ-MSCs (E, F), PL-MSCs (G, H) and AM-MSCs (I, J). Osteogenic differentiation was evidenced by the formation of alkaline phosphatase-positive aggregates in cytoplasm after osteogenic induction using NH OsteoDiff Medium (B, D, F, H, J, respectively). No alkaline phosphatase-positive aggregates was found in cytoplasm of BM-MSCs, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs cultured in DMEM supplemented with 10% FBS (A, C, E, G, I, respectively).

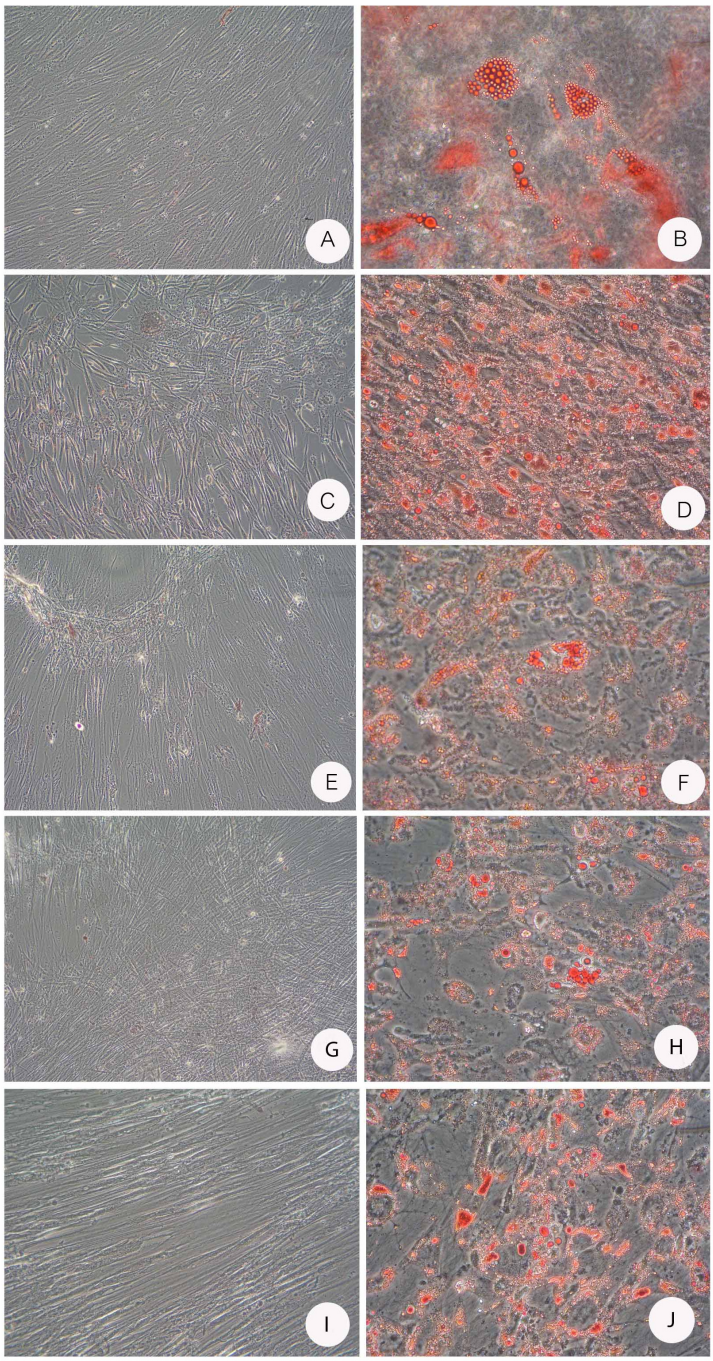

Figure 4.15 Representative photomicrographs of adipogenic differentiation of BM-MSCs (A, B), UC-MSCs (C, D), WJ-MSCs (E, F), PL-MSCs (G, H) and AM-MSCs (I, J). Adipogenic differentiation was evidenced by the formation of lipid droplet (oil red-O-positive) in cytoplasm after adipogenic induction using NH AdipoDiff Medium (B, D, F, H, J, respectively). No lipid droplet was observed in the cytoplasm of BM-MSCs, UC-MSCs, WJ-MSCs, PL-MSCs and AM-MSCs cultured in DMEM supplemented with 10% FBS (A, C, E, G, I, respectively).

Immunomudulatory effect of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs in comparison to BM-MSCs

To determine whether MSCs can inhibit the proliferation of alloreactive T-lymphocyte, the responder PB-MNCs were labeled with CFSE. CFSE is a fluorescent dye which can pass through the cell membrane and generated a fluorescent hydrophilic metabolite readily detectable by flow cytometer. Because CFSE concentration decreases by half after each cell division, it can provide a history of the proliferation of each labeled cells. Fig. 4.16 is a representative result showing the gating of cells used to calculate the proliferation index of labeled cells.

The immunomudulatory effect of UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs compared to that of BM-MSCs as determined by MLR assays is summarized in Table 16, Fig. 4.17 UC-MSCs, WJ-MSCs, PL-MSCs, AM-MSCs and BM-MSCs significantly reduced the proliferation of alloreactive T-lymphocytes compared to controls MLR without MSCs addition ($P < 0.05$) (Fig. 4.17). By comparison between sources, PL-MSCs and AM-MSCs possessed higher immunosuppressive capacity than BM-MSCs, while UC-MSCs and WJ-MSCs exhibited lower immunosuppressive capacity than BM-MSCs. However, the differences in the immunosuppressive capacity among those MSCs were not statistical significant ($P> 0.05$).

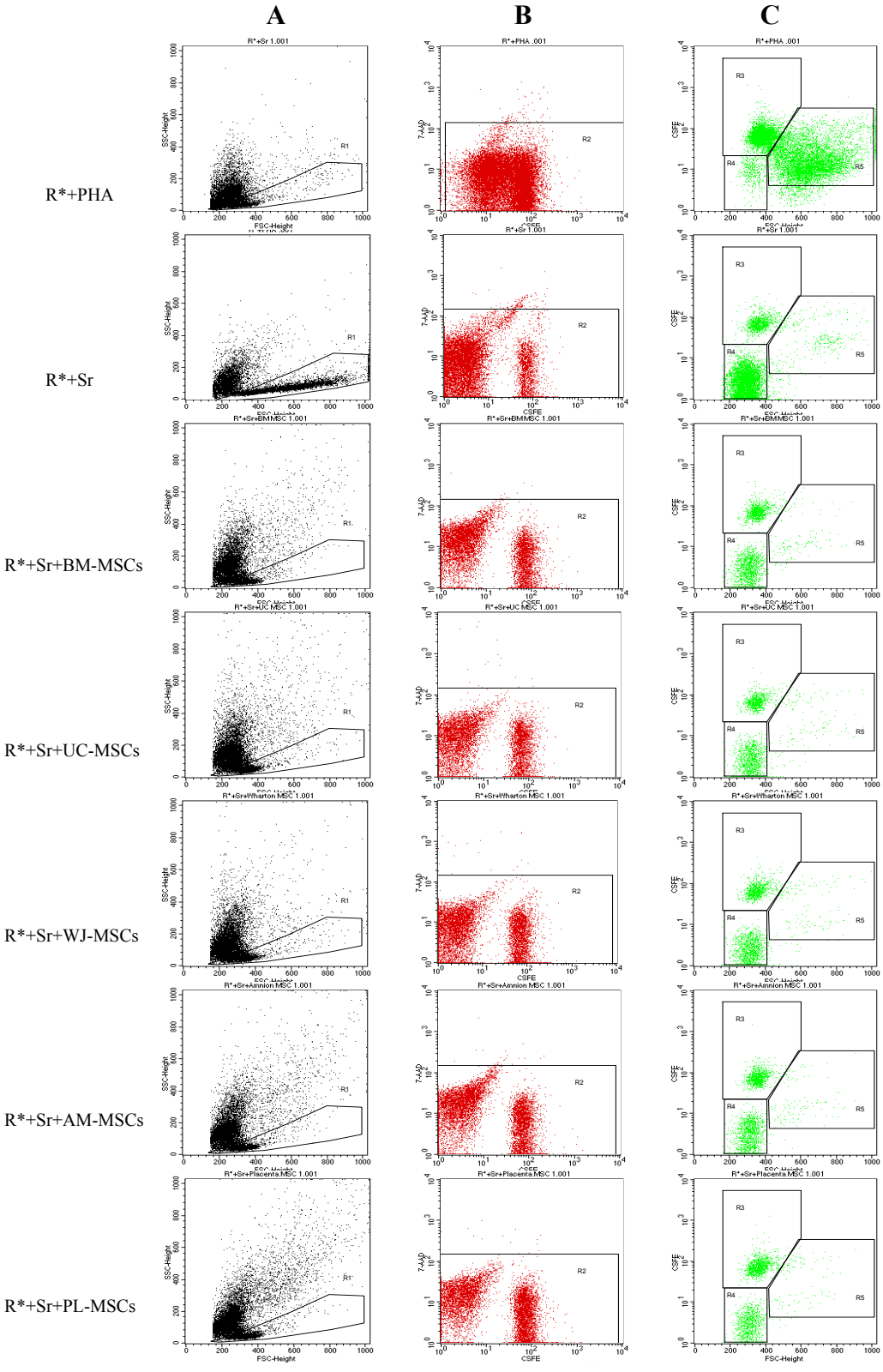

Figure 4.16 MLR assays were analyzed by flow cytometer. A: Dot plot of forward scatter and side scatter show the T-lymphocyte fraction of peripheral blood mononuclear cells. B: Dot plot of CSFE and 7-ADD show the living T-lymphocytes with CFSE-positive. C: Dot plot of CSFE and forward scatter show T-lymphocytes labeled with CFSE dividing in response to irradiated PB-MNCs.

Table 10

The proliferation of alloreactive T-lymphocytes (case 1)
co-cultured with MSCs from various sources

	Experiment 1			Experiment 2			Experiment 3			
MRI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	**PI***
R*+R	31.62	1.43	4.32	30.88	1.57	4.83	35.59	1.75	4.68	**4.61**
R*+PHA	48.14	41.36	46.21	50.96	38.29	42.9	48.14	41.36	46.21	**45.11**
R*+Sr	14.67	12.45	45.9	13.19	10.93	45.31	12.48	12.42	49.87	**47.03**
R*+Sr+BM MSC	35.77	4.01	10.08	45.95	3.39	6.87	35.54	3.95	10	**8.98**
R*+Sr+UC MSC	37.12	4.87	11.59	33.6	3.49	9.4	32.19	4.25	11.63	**10.87**
R*+Sr+WJ MSC	31.43	3.97	11.21	31.46	4.22	11.82	35.24	4.57	11.47	**11.50**
R*+Sr+AM MSC	38.82	4.33	10.03	38.07	3.96	9.42	38.07	3.96	9.42	**9.62**
R*+Sr+PL MSC	47.54	2.97	5.88	49.27	4.15	7.75	51.69	3.44	6.23	**6.62**

* Values are presented as mean ± SEM

R: Responder cells

R*: CFSE-labeled responder cells

Sr: Irradiated stimulator cells

Table 11

The proliferation of alloreactive T-lymphocytes (case 2)
co-cultured with MSCs from various sources

	Experiment 1			Experiment 2			Experiment 3			
MRI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	**PI***
R*+R	50.6	1.23	2.37	51.57	1.59	2.99	51.57	1.27	2.4	**2.59**
R*+PHA	43.12	46.78	52.03	43.64	45.92	51.27	42.9	46.76	52.15	**51.82**
R*+Sr	12.43	12.29	49.71	12.8	10.16	44.25	13.57	9.75	41.8	**45.25**
R*+Sr+BM MSC	37.35	4.64	11.05	36.52	4.49	10.94	37.31	4.46	10.67	**10.89**
R*+Sr+UC MSC	32.38	5.35	14.17	34.57	5.43	13.57	33.98	5.29	13.47	**13.74**
R*+Sr+WJ MSC	32.6	5.06	13.43	28.84	4.65	13.88	30.3	5.11	14.43	**13.91**
R*+Sr+AM MSC	41.43	4.57	9.93	39.73	4.68	10.53	41.33	4.28	9.38	**9.95**
R*+Sr+PL MSC	49.97	4.93	8.97	50.34	4.73	8.58	51.3	3.71	6.74	**8.10**

* Values are presented as mean ± SEM

R: Responder cells

R*: CFSE-labeled responder cells

Sr: Irradiated stimulator cells

Table 12

The proliferation of alloreactive T-lymphocytes (case 3)
co-cultured with MSCs from various sources

	Experiment 1			Experiment 2			Experiment 3			
MRI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	**PI***
R*+R	75.38	3.5	4.43	75.02	3.21	4.1	77.08	3.04	3.79	**4.11**
R*+PHA	50.2	47.45	48.59	52.93	46.21	46.61	49.51	45.26	47.75	**47.65**
R*+Sr	13.21	11.78	47.13	12.46	10.28	45.2	14.13	12.45	46.91	**46.41**
R*+Sr+BM MSC	41.25	5.58	11.91	48.47	6.69	12.12	51.23	7.12	12.2	**12.08**
R*+Sr+UC MSC	39.85	4.78	10.71	41.59	5.21	11.13	42.56	4.14	8.86	**10.23**
R*+Sr+WJ MSC	36.54	3.21	8.07	40.12	4.15	9.37	38.46	4.08	9.59	**9.01**
R*+Sr+AM MSC	41.57	4.19	9.15	50.16	6.14	10.9	48.85	5.53	10.16	**10.07**
R*+Sr+PL MSC	50.14	4.11	7.57	51.26	5.46	9.62	54.21	5.89	9.8	**9.00**

* Values are presented as mean ± SEM

R: Responder cells

R*: CFSE-labeled responder cells

Sr: Irradiated stimulator cells

Table 13

The proliferation of alloreactive T-lymphocytes (case 4)
co-cultured with MSCs from various sources

	Experiment 1			Experiment 2			Experiment 3			
MRI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	**PI***
R*+R	58.71	1.17	1.95	58.34	2.38	3.91	57.84	2.43	4.03	**3.30**
R*+PHA	38.13	34.73	47.66	37.86	31.67	45.54	39.54	35.51	47.31	**46.84**
R*+Sr	12.19	9.65	44.18	13.64	10.11	42.56	13.66	9.99	42.24	**42.99**
R*+Sr+BM MSC	40.12	5.11	11.29	37.54	6.2	14.17	39.87	5.51	12.14	**12.53**
R*+Sr+UC MSC	39.61	5.54	12.27	40.16	4.21	9.48	42.13	4.25	9.16	**10.30**
R*+Sr+WJ MSC	39.78	4.11	9.36	38.85	4.05	9.44	40.12	4.28	9.63	**9.48**
R*+Sr+AM MSC	40.44	4.28	9.57	41.02	4.54	9.96	43.24	4.37	5.85	**8.46**
R*+Sr+PL MSC	46.96	3.69	7.28	48.88	3.35	6.41	49.03	4.12	7.75	**7.15**

* Values are presented as mean ± SEM

R: Responder cells

R*: CFSE-labeled responder cells

Sr: Irradiated stimulator cells

Table 14

The proliferation of alloreactive T-lymphocytes (case 5)
co-cultured with MSCs from various sources

	Experiment 1			Experiment 2			Experiment 3			
MRI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	Gated R3 (%)	Gated R5 (%)	PI	**PI***
R*+R	65.67	5.12	7.23	66.46	3.98	5.65	59.65	4.42	6.89	**6.59**
R*+PHA	40.15	38.87	49.19	40.35	37.22	47.98	39.78	35.64	47.25	**48.14**
R*+Sr	12.8	9.87	43.53	15.79	10.11	39.03	14.57	10.21	41.2	**41.25**
R*+Sr+BM MSC	45.58	5.48	10.73	44.83	5.57	11.05	41.41	4.89	10.56	**10.78**
R*+Sr+UC MSC	41.25	5.77	12.27	42.64	4.98	10.45	40.45	6.02	12.95	**11.89**
R*+Sr+WJ MSC	39.81	4.27	9.68	41.48	4.36	9.51	43.2	5.57	11.42	**10.20**
R*+Sr+AM MSC	37.99	2.15	5.35	39.92	4.16	9.43	41.93	3.77	8.24	**7.67**
R*+Sr+PL MSC	41.79	3.65	8.03	43.67	3.85	8.1	39.41	3.8	8.79	**8.31**

* Values are presented as mean ± SEM

R: Responder cells

R*: CFSE-labeled responder cells

Sr: Irradiated stimulator cells

Table 15

Mean value of proliferation index of alloreactive T-lymphocytes co-cultured with MSCs from various sources.

MLR	PI (%)±SEM	*p*-value
R*+R	4.24±1.52	Negative control
R*+PHA	47.91±2.47	Positive control
R*+Sr	44.58±2.42	-
R*+Sr+BM-MSCs	11.05±1.38	-
R*+Sr+UC-MSCs	11.40±1.46	0.75
R*+Sr+WJ-MSCs	10.82±1.97	0.83
R*+Sr+AM-MSCs	9.15±1.05	0.09
R*+Sr+PL-MSCs	7.8±0.93	0.06

Values are presented as mean ± SEM.

* $p<0.05$ significantly different compared to control (R*+Sr+BM-MSCs)

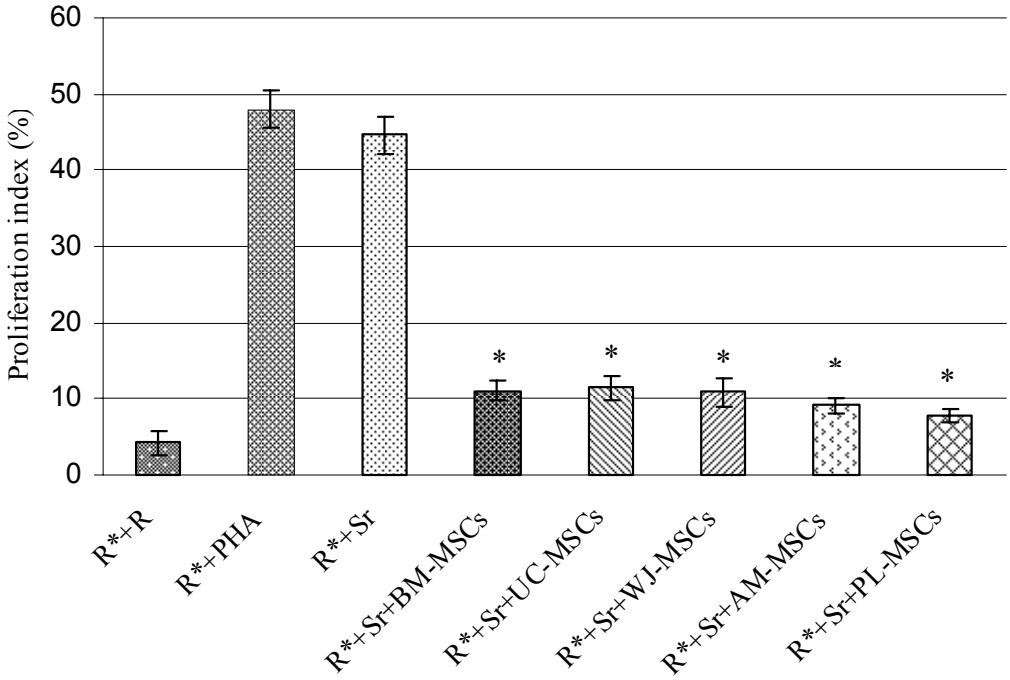

Figure 4.17 Mean value of proliferation index of CFSE labeled responder T-lymphocytes co-cultured with irradiated stimulator T-lymphocytes and MSCs from various sources. Data are presented as mean ± SEM. *P<0.05: significantly different compared to control (R* + Sr).