Superiority of Adipose-derived CD34+ Cells over Adipose-derived Stem Cells in Promoting Ischamic Tissue Survival

Yan-Jun Liu
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

Tian-Yu Zhang
Shanghai Normal University

Poh-Ching Tan
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

Yun Xie
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

Pei-Qi Zhang
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

Yi-Ming Gao
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

Xiao-Jie Zhang
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

Shuang-Bai Zhou (shuangbaizhou@yahoo.com)
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery https://orcid.org/0000-0001-5934-1991

Qing-Feng Li
Shanghai 9th Peoples Hospital Affiliated to Shanghai Jiaotong University School of Medicine Department of Plastic Surgery

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Research

Keywords: Adipose-derived CD34+ cells, Tissue ischemia, Inflammation, Angiogenesis

Posted Date: March 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-336040/v1

Abstract

Background: Tissue ischemia usually leads to necrosis and is a threatening condition associated with reconstructive surgery. Promoting the survival of ischemic tissue is critical for improving clinical outcomes. Although various solutions based on stem cells have been reported, there are still limitations to clinical translation. The aim of this study was to develop an effective method to promote the survival of ischemic tissue.

Methods: Adipose-derived CD34+ and CD34- cells were obtained by magnetic bead sorting from the stromal vascular faction (SVF). Adipose-derived stem cell (ADSC) were collected by subculture. The angiogenic capacities of CD34+ cells, CD34- cells and ADSC were evaluated in vitro by comparing mRNA and protein expression. Random axial flaps in nude mice were used to evaluate the efficacy of these cells in protecting tissue from necrosis. The effect of these cells in preventing inflammation was also evaluated.

Results: Our data suggest that CD34+ cells expressed higher levels of angiogenetic factors and lower levels of inflammatory factors than the other cell types. More vessel branches were formed when human umbilical vein endothelial cells (HUVECs) were treated with conditioned medium from CD34+ cells than conditioned medium from the other cell types. Compared to ADSC, CD34+ cells showed significantly higher efficacy in promoting tissue survival. More CD31+ cells and higher levels of angiogenic factors were observed in tissues from the CD34+ Group than from the other Groups. Lower levels of the proinflammatory factors TNF-α and IL-1b and higher levels of anti-inflammatory factors were found in the CD34+ Group than in the other Groups.

Conclusion: Adipose-derived CD34+ cells showed better efficacy in improving ischemic tissue survival than ADSC by reducing tissue inflammation and promoting angiogenesis. CD34+ cells can be obtained easily and may be suitable for clinical applications.

1. Background

Soft tissue defects are one of the most common diseases in the field of reconstruction. Skin flaps are the most widely used method to reconstruct defects. However, the blood supply of skin flaps is unreliable and can negatively impact the outcome. Insufficient blood supply results in tissue ischemia, which is accompanied by inflammation and leads to partial or even total necrosis of the flap [1, 2]. In recent years, therapeutic strategies to promote flap survival in clinical settings have been actively studied.

Researchers have investigated using drugs or physical methods to improve the survival of skin flaps. However, these methods are not suitable for clinical applications because of toxicity, limited maintenance time or other factors [3].

Adipose tissue and its enriched adipose-derived stem cell (ADSC) show advantages in promoting tissue repair and regeneration [4, 5]. However, studies have shown that during culture, ADSC lose the surface

marker CD34, which is an important surface marker expressed on freshly isolated stem cells [6]. CD34 was first identified on the surface of a variety of stem cells and progenitor cells, such as hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) [7]. After injury, CD34+ cells can be rapidly induced, recruited to the injury site and participate in vascular repair and angiogenesis [8-10]. Previous research showed that high endogenous expression of CD34 on ADSCs had a significant beneficial effect on improving the outcome of human fat grafting [11]

Stromal vascular fractions (SVFs) are highly heterogeneous populations of cell hybrids obtained after the digestion of adipose tissue and contain both CD34+ ADSC and other cell subpopulations expressing CD34, such as EPCs and HSCs [12-14]. These cells have been reported to contribute to vascularization [15, 16]. We hypothesized that adipose-derived CD34+ cells will show better efficacy in promoting tissue angiogenesis than ADSC. This study aimed to compare the effect of adipose-derived CD34+ cells and ADSC on promoting ischemic tissue survival and discuss the underlying mechanism.

2. Materials And Methods

2.1 Adipose tissue collection

Subcutaneous abdominal adipose tissues were obtained from 4 healthy female donors aged 20-40 years who underwent abdominal liposuction. The donors all provided informed consent. This study was approved by the Ethics Committee of Shanghai Ninth People's Hospital and complied with the principles of the Declaration of Helsinki. The SVFs were obtained by collagenase digestion as previously described [17].

2.2 Magnetic bead sorting and purity assay

Magnetic bead separation kits (Miltenyi Biotec, Germany) were used to sort CD34+ and CD34- cells. Briefly, 1×10^8 cells were treated with CD34 magnetic beads at 4°C for 30 min, and a suitable amount of phosphate-buffered saline (PBS) was added for the centrifugation step. CD34+ and CD34- cells were separated by a magnetic bead separation column. The purity of CD34+ (1×10^6 cells/100 μl) was analyzed by a FACS Aria flow cytometer (Becton-Dickinson, San Jose, CA, USA).

2.3 Immunophenotypic analysis

For flow cytometric analysis, Passage 4 (P4) CD34+ were isolated by magnetic beads and incubated with monoclonal antibodies against CD73 (APC, clone 555479), CD90 (PE, clone 555479), CD105 (PE, clone 555443), HLA-DR (FITC, clone 555441), CD34 (FITC, clone 555822) and CD45 (PE, clone 555446). All antibodies were purchased from BD Biosciences. The cells were subsequently washed with PBS, fixed with 4% formaldehyde and analyzed on a FACS Aria flow cytometer.

2.4 Tube formation assay

Human umbilical vein endothelial cells (HUVECs) were purchased from the ATCC and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in a 37°C humidified incubator containing 5% CO2. When the density of the HUVECs reached 90%, the cells were digested and passaged with 0.25% trypsin-EDTA (Sigma, St. Louis, MO, USA). Precooled Matrigel (50 μl per well) was added to a 96-well plate and incubated for 30 min. CD34+ cells, CD34- cells and ADSC (P4) were cultured for 24 hours, and the culture medium was collected and centrifuged at 1700 rpm for 5 min, and the supernatant was used as conditioned medium (CM). The HUVECs were adjusted to 6×10^5 cells/ml and centrifuged, and 50 μl of CM (CD34+-CM, CD34–-CM and ADSC-CM) was added. The formation of tubes was observed under a microscope every two hours using a Labovert phase contrast microscope (Leitz, Los Angeles, CA, USA), and images were captured with an attached digital camera (Pixera, Santa Clara, CA, USA).

2.5 Animal model

Thirty-six 6- to 8-week-old male nude mice were purchased from Shanghai Laboratory Animal Center (SLAC). The mice were anesthetized by intraperitoneal injection of 10% glutaraldehyde at a dose of 3.5 ml/kg. A skin flap (5 cm × 0.8 cm) with the pedicle located between the ears was created along the long axis of the body on the backs of the mice. The flap was separated from the deep fascia. All blood vessels were electrocoagulated while exposed, and blood vessels were supplied to the pedicle and suture with 5-0 prolene. All mice were randomly divided into four groups: the control group (n=9, each mice was inject 0.1 ml PBS), CD34+ Group (n=9, each mouse was injected with 0.1 ml, 5×10^6 CD34+ cells/ml), ADSC Group (n=9, each mouse was injected with 0.1 ml, 5×10^6 ADSCs/ml) and CD34- Group (n=9, each mouse was injected with 0.1 ml, 5×10^6 CD34- cells/ml). All animal studies were approved by the Animal Research Committee of Shanghai Ninth People's Hospital.

2.6 Flap necrosis rate evaluation

On the 7th day after model establishment (D7), high-quality photographs of the flaps were captured to evaluate the flap necrosis rate with ImageJ software. The percentages of the necrotic areas were calculated as follows: necrotic area/total flap area×100%.

2.7 H&E and Masson’s trichrome staining

Specimens of distal tissue 3-4 from each flap were harvested on D7. The samples were first fixed in 4% paraformaldehyde for 24 hours and embedded in paraffin. Sections with a thickness of 4 μm were prepared and mounted on poly-l-lysine-coated slides for H&E and Masson’s trichrome staining, as previously described [18]. Light microscopic examination (Olympus Corporation, Tokyo, Japan) and analysis were performed on six random fields of three random sections from each tissue specimen.

2.8 Enzyme-linked immunosorbent assay (ELISA)

The CM of CD34+ cells, CD34- cells and ADSC (P4) was collected, and the supernatants were used. The concentrations of cytokines such as VEGF, bFGF, TGF-β and IL-10 were measured by ELISA kits from R&D Systems (Minneapolis, MN, USA) in accordance with the manufacturer's instruction in 96-half-well Maxisorp plates. Cytokine binding was developed with 3,3',5,5'-tetramethylbenzidine (TMB) and stopped with 1 M sulfuric acid. The absorbance was measured at 450 nm on a standard ELISA reader from BioTek (Bad Friedrichshall).

2.9 RNA isolation and real-time polymerase chain reaction(RT-PCR)

TRIzol reagent (Invitrogen, Mulgrave, Australia) was used to extract the total RNA. The RNA was reverse transcribed into complementary DNA by using RevertAid reverse transcriptase (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. RT-PCR was performed with SYBR Premix EX Taq (Takara, Dalian, China) by using a ViiA 7 (Life Technologies, Carlsbad, CA). The housekeeping gene β-actin was used for normalization.

2.10 Western blot analysis

Total proteins were extracted from cells with radioimmunoprecipitation assay (RIPA) lysis buffer. After determining the protein concentrations by a bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Waltham, MA), twenty micrograms total protein were separated by 10% SDS-PAGE and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). The membranes were blocked with 5% bovine serum albumin (BSA) and then probed with primary antibodies against VEGF, bFGF and SDF-1 (1:1000; all from Abcam, Cambridge, UK). The blots were then incubated with HRP-conjugated secondary antibodies and visualized using an enhanced chemiluminescence detection system (Millipore, Bedford, MA). Quantitative analysis of the immunoreactive bands was conducted by ImageJ software.

2.11 Immunofluorescence staining

Skin sections were incubated with primary antibodies against CD31 or CD68 (all from Abcam, Cambridge, United Kingdom) at 4°C overnight and then visualized using Alexa Fluor 555 secondary antibodies. Fluorescence staining was examined using a confocal laser scanning fluorescence microscope (Carl Zeiss, Jena, Germany). Quantitative analyses were performed by average optical (AO) analysis. The positive expression level was directly proportional to the AO value. AO=IOD /AREA, where IOD represents the cumulative optical density and AREA represents the area of the selected region.

2.12 Statistical analysis

The results are presented as the mean ± SD. Statistical differences among groups were assessed using one-way ANOVA and two-tailed Student's t tests. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 ADSC lost CD34 expression after subculture.

We analyzed the expression of CD34 in freshly isolated SVF cells and subcultured P4 ADSC. The results showed that 59.1± 0.35% of freshly isolated SVF cells expressed the surface marker CD34. After culture, only 0.3% of cells expressed CD34 (Figure 1).

3.2 Adipose-derived CD34+ cells exhibited better proangiogenic capacities than ADSCs in vitro.

We assessed the effect of CD34+ cells, ADSC and CD34- cells in promoting HUVEC tube formation. The results showed that although the total branch lengths of tubes formed in the ADSC Group (32749.33 ± 1156.50 μm/field) were longer than those in the CD34- and control Groups, they were still significantly shorter than those in the CD34+ Group (37093.67 ± 1992.44 μm/field, p= 0.030) (Figure 2A-B). These results indicated that CD34+ cells had better proangiogenic capacities than ADSC.

3.3 Adipose-derived CD34+ cells expressed higher levels of proangiogenic factors than ADSC in vitro.

The mRNA expression of VEGF, bFGF and SDF-1 was 6.75 ± 0.24, 5.07 ± 0.15, and 4.06 ± 0.23, respectively, in CD34+ cells (Figure 2D). These results showed that the expression of the proangiogenic genes SDF-1, VEGF, and bFGF in CD34+ cells was significantly higher than that in the other Groups. The ELISA results also showed that the secretion of the proangiogenic factors VEGF and bFGF in CD34+-CM (460.13 ± 54.40 pg/ml/10^5 cells, 398.14 ± 27.15 pg/ml/10^5 cells, respectively) was significantly higher than that in ADSC-CM and CD34–CM (Figure 2C).

3.4 Adipose-derived CD34+ cells expressed higher levels of anti-inflammatory factors and lower levels of proinflammatory factors than ADSC in vitro.

We evaluated the expression of the inflammation-related factors IL-10, TGF-β, TNF-α, and IL-1b by RT-PCR. Compared with those in the ADSC Group, the mRNA expression levels of the anti-inflammatory factors IL-10 and TGF-β, in the CD34+ Group were 3.08 ± 0.30 and 3.67 ± 0.28, respectively, which were significantly higher than those in the ADSC Group (1.81 ± 0.26, p=0.003, 2.44 ± 0.30, p=0.032, respectively). The expression of the proinflammatory factors TNF-α and IL-1b was lower in the CD34+ Group (0.40 ± 0.02, 0.29 ± 0.06, respectively) than in the ADSC Group (0.56± 0.03, p=0.001; 0.49± 0.01, p=0.009, respectively) (Figure 3A). The ELISA results also verified these differences. The secretion of the inflammation-related factors TGF-β and IL-10 in CD34+-CM (148.36 ± 24.43 pg/ml/10^5 cell, 124.07 ± 25.36 pg/ml/10^5 cell, respectively) was significantly higher than that in ADSC-CM and CD34–CM (Figure 3B).

3.5 Adipose-derived CD34+ cells induced better outcomes than ADSC in reducing the necrosis area rates of ischemic tissue.

We used random axial flaps in nude mice to evaluate the efficacy of adipose-derived CD34+ cells and ADSC in protecting tissue from necrosis. The necrosis area rates of ischemic skin flaps in the CD34+ Group (19.70 ± 1.50%) were significantly lower than those in the ADSC Group (30.30 ± 3.50%, p= 0.008),

CD34- Group (52.70 ± 7.50%, p= 0.002) and PBS Group (71.70 ± 7.60, p< 0.001) (Figure 4A-B). H&E staining showed improved tissue construction and reduced inflammatory cell infiltration in the CD34+ Group. Masson staining showed more collagen deposition in the CD34+ Group than in the other Groups (Figure 4C). These results demonstrated that CD34+ cells were more efficient in protecting ischemic tissue from necrosis.

3.6 Adipose-derived CD34+ cells exhibited better proangiogenic potential than ADSC in vivo.

Vessel density was evaluated by anti-CD31 histochemical staining of tissue sections collected on day 7. Tissues from both the CD34+ Group and the ADSC Group showed higher CD31+ cell density than those from the CD34- Group and the control Group. When compared to each other, the CD34+ Group was significantly superior to the ADSC Group regarding CD31+ cell density (0.33 ± 0.02 vs 0.26 ± 0.02, p=0.009) (Figure 5A-B).

3.7 Adipose-derived CD34+ cells expressed higher levels of proangiogenic genes and proteins than ADSC in vivo.

We subsequently evaluated the expression of the angiogenesis-related factors SDF-1, VEGF and bFGF. Compared with that in the ADSC Group, the mRNA expression of the proangiogenic factors SDF-1, VEGF and bFGF in tissues from the CD34+ Group (4.06 ± 0.23,3.52 ± 0.27 and 4.96 ± 0.47, respectively) were significantly higher than those in tissues from the ADSC Group and CD34- Group (all p□0.05) (Figure 5C). The protein expression levels of SDF-1, VEGF and bFGF were also higher in tissues from the CD34+ Group than in those from the other Groups (Figure 5D-E). Previous studies reported that SDF-1 could promote EPCs migration and proliferation via the PI3K/AKT signaling pathway. Our results showed that the expression of PI3K and AKT was increased in tissues from the CD34+ Group (p= 0.002 and p= 0.019, respectively) (Figure 5F), supporting the superior angiogenic efficacy of CD34+ cells.

3.8 Adipose-derived CD34+ cells induced less immune cell infiltration than ADSC in vivo.

We assessed immune cell infiltration in the different experimental Groups by anti-CD68 immunohistochemical staining. The results showed fewer CD68+ macrophages in the CD34+ Group (0.067±0.061) than in the ADSC Group (0.17 ± 0.03, p= 0.04) or the CD34- Group (0.26 ± 0.02, p< 0.01) (Figure 6A-6B). These results indicated that tissues from the CD34+ Group exhibited lower inflammation levels than those from the ADSC Group.

3.9 Adipose-derived CD34+ cells expressed lower levels of inflammation than ADSC in vivo.

Tissues from the CD34+ Group expressed higher levels of IL-10 and TGF-β mRNA and protein than those from the ADSC Group or the CD34- Group (all p< 0.05) (Figure 6C, E). Consistent with the in vitro results, the expression levels of TNF-α and IL-1b were reduced in the CD34+ Group (5.56 ± 0.39 and 0.23 ± 0.02) (Figure 6D-E). Studies have shown that the p38 MAPK/NF-κB pathway initiates a cascade reaction via phosphorylation, resulting in the release of TNF-α and IL-1β and promoting inflammatory reactions [19, 20].

Our results showed that the expression of p38 MAPK and NF-κB was lower in tissues from the CD34+ Group than in those from the ADSC Group (Figure 5F), indicating reduced inflammation in the presence of CD34+ cells.

4. Discussion

Random flaps are one of the most commonly used methods to repair soft tissue defects caused by trauma or tumor ablation [21]. However, ischemia and necrosis are the most serious complications associated with the repair process. Previous studies have shown that intraperitoneal injection of icariin or subcutaneous injection of hirudin and other drugs can improve the survival rates of skin flaps, but clinical translation of these methods is poor [22, 23]. In recent years, autologous stem cells such as ADSC have been widely studied and applied to promote tissue regeneration and repair, but there are shortcomings in this treatment strategy. ADSC lose the surface marker CD34, which is an important marker on freshly isolated stem cells and may impact the efficacy of this treatment [24].

CD34 was first identified on HSCs and then on a series of other progenitor cells [25-27]. Previous studies have shown that CD34+ bone marrow cells can respond to tissue injury quickly, mobilizing from the bone marrow into the peripheral blood, migrating to the injured site and participating in angiogenesis. It has been reported that newly isolated ADSC express CD34. After culture, ADSC lose the marker CD34 [28]. Moreover, CD34+ cells in adipose tissue contain M2 macrophage subpopulations that express IL-10 and other immunoregulatory genes. Transplantation of adipose-derived CD34+ cells has been reported to reduce inflammation in myocardial infarction. Hence, we hypothesized that CD34+ cells isolated from SVFs may be superior to ADSCs in promoting the survival of ischemic tissue.

We used random axial flaps in nude mice to evaluate the efficacy of adipose-derived CD34+ cells and ADSC in protecting tissue from necrosis. Both CD34+ cells and ADSC showed significant effects in promoting tissue survival compared to CD34- cells. When compared to each other, the survival rate in the CD34+ Group was significantly higher than that in the ADSC Group. Histological staining showed more collagen deposition and less inflammatory cell infiltration in the CD34+ Group than in the ADSC Group, indicating that the tissue structure was better preserved in the CD34+ Group.

As the ex vivo results showed, the protective effect of adipose-derived CD34+ cells may involve two mechanisms: promoting vascularization and ameliorating inflammation.

Vascularization is vital to ischemic tissue [20]. Histological analysis of ischemic skin showed that tissues from the CD34+ Group contained more CD31+ cells than those from the ADSC Group. ADSC have been reported to have angiogenic effects by secreting VEGF, bFGF and other growth factors [29, 30]. In our ex vivo study, we found that adipose-derived CD34+ cells expressed higher levels of VEGF and bFGF mRNA than ADSCs, as well as the related secreted proteins. The in vivo results showed that higher VEGF and bFGF expression at both the mRNA and protein level was observed in skin tissue harvested from the CD34+ Group. Moreover, we found that SDF-1 was significantly more highly expressed in CD34+ cells

than in cultured ADSC. SDF-1 is an important chemokine that can induce cell migration and promote angiogenesis [31, 32]. The expression of SDF-1 was also significantly higher in tissues from the CD34+ Group than in those from the ADSC Group. SDF-1 can promote EPCs migration and proliferation via the PI3K/AKT signaling pathway. In HUVECs, the CM of CD34+ cells promoted more capillary tube formation than that of ADSC, indicating that adipose-derived CD34+ cells had better angiogenic effect than ADSC.

Reduced immune cell infiltration was observed in skin sections from the CD34+ group. Fewer CD68+ macrophages were found in the CD34+ Group than in the other Groups. Ischemia induces the secretion of inflammatory factors, leading to inflammatory activation and worsening tissue necrosis. Previous studies have reported that ADSC can ameliorate inflammation [33]. In our study, CD34+ cells secreted higher levels of the anti-inflammatory factors IL-10 and TGF-β and lower levels of the proinflammatory factors TNF-α and IL-1b than ADSC. We also found that the expression of p38 MAPK and NF-κB was reduced in tissues from the CD34+ Group. The p38 MAPK/NF-κB pathway initiates a cascade reaction via phosphorylation, resulting in the release of proinflammatory cytokines [19, 20]. CD34+ M2 macrophages, which play a role in regulating inflammation [34], may contribute to this effect. Therefore, adipose-derived CD34+ cells had a better effect on ameliorating ischemia-induced inflammation than the other cells.

Compared with ADSC, CD34+ cells have the advantages of rapid isolation and no need for long-term in vitro culture, showing promising prospects for clinical transformation. Our research demonstrated that adipose-derived CD34+ cells show better efficacy in promoting the survival of ischemic tissue than ADSC. However, this study also has some limitations. This study assessed the effect of CD34+ cells on the survival of random axial flaps using nude mice. Clinical trials are needed to evaluate the clinical efficacy of adipose-derived CD34+ cells.

5. Conclusion

Adipose-derived CD34+ cells showed better efficacy in promoting the survival of ischemic tissue than ADSC by enhancing vascularization and reducing inflammation. As there is no need for ex vivo culture, adipose-derived CD34+ cells can be a promising tool for clinical applications.

Abbreviations

ADSC: adipose-derived stem cell

BSA: bovine serum albumin

EPCs: endothelial progenitor cells

HSCs: hematopoietic stem cells

HUVECs: human umbilical vein endothelial cells

MSCs: mesenchymal stem cells

PVDF: polyvinylidene difluoride

Declarations

Ethics approval and consent to participate

The donors of abdominal subcutaneous adipose tissues all provided informed consent. This study was approved by the Ethics Committee of Shanghai Ninth People's Hospital and complied with the principles of the Declaration of Helsinki. Consent to participate is not applicable.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by National Natural Science Foundation of China (81971848; 81620108019); Clinical Research Plan of SHDC (SHDC2020CR1019B; SHC2020CR402); Shanghai Municipal Key Clinical Specialty (shslczdzk00901); Innovative research team of high-level local universities in Shanghai (SSMU-ZDCX20180700)

Authors' contributions

YJL, SBZ and QFL initiated and designed the study and protocol. SBZ and YX recruited all patients. YJL, TYZ and PCT participated in the data collection and data analysis. PCT, XJZ, PQZ and YMG contributed to the data interpretation. TYZ and PCT wrote the first draft of the manuscript, and QFL and SBZ critiqued and modified the manuscript. All authors reviewed and approved the work.

Acknowledgements

The authors gratefully acknowledge the technology support from Core Facility of Basic Medical Sciences, Shanghai Jiao Tong University School of Medicine. We thank American Journal Experts (AJE) for editing the English language of the manuscript.

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Figures

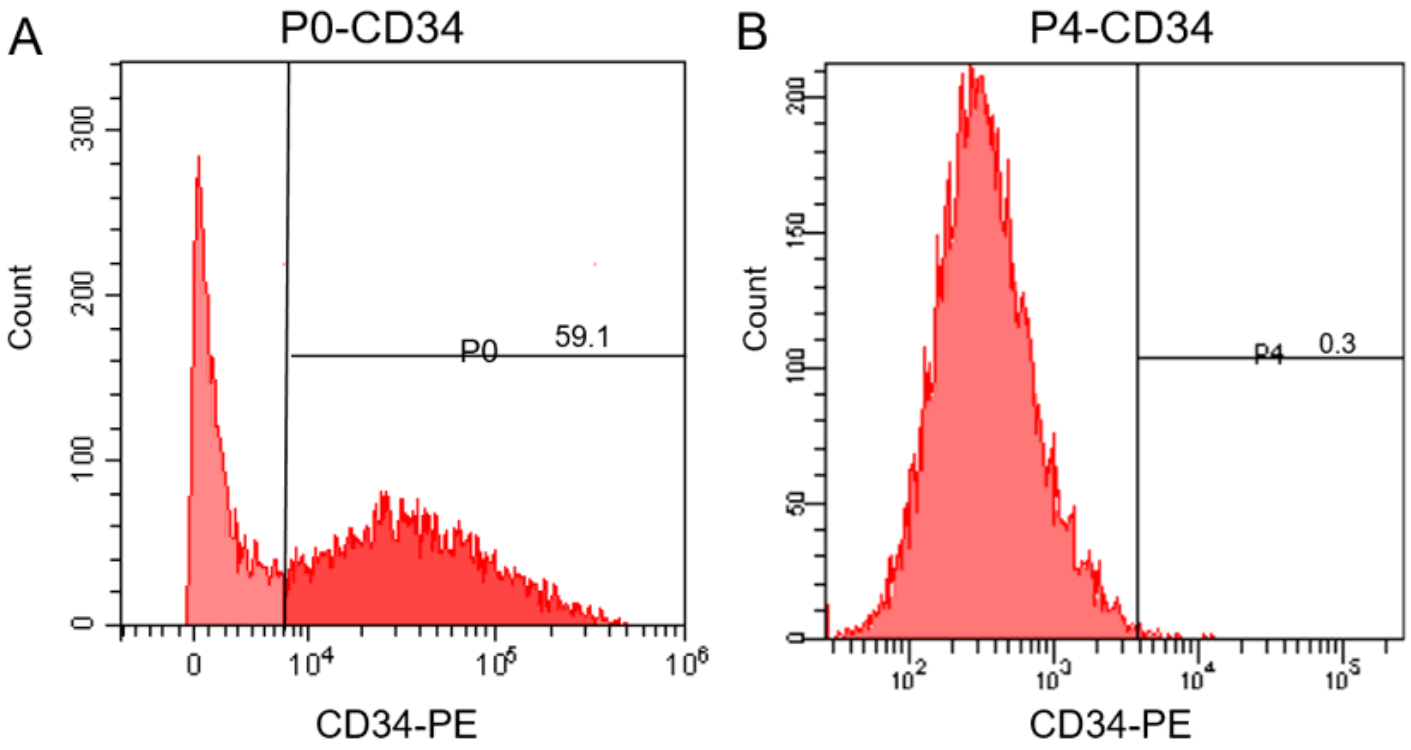

Figure 1

Immunophenotypic analysis of human adipose-derived CD34+ cells. (A) The expression of CD34 in P0 adipose-derived CD34+ cells by flow cytometry. (B) The expression of CD34 in P4 adipose-derived CD34+ cells by flow cytometry.

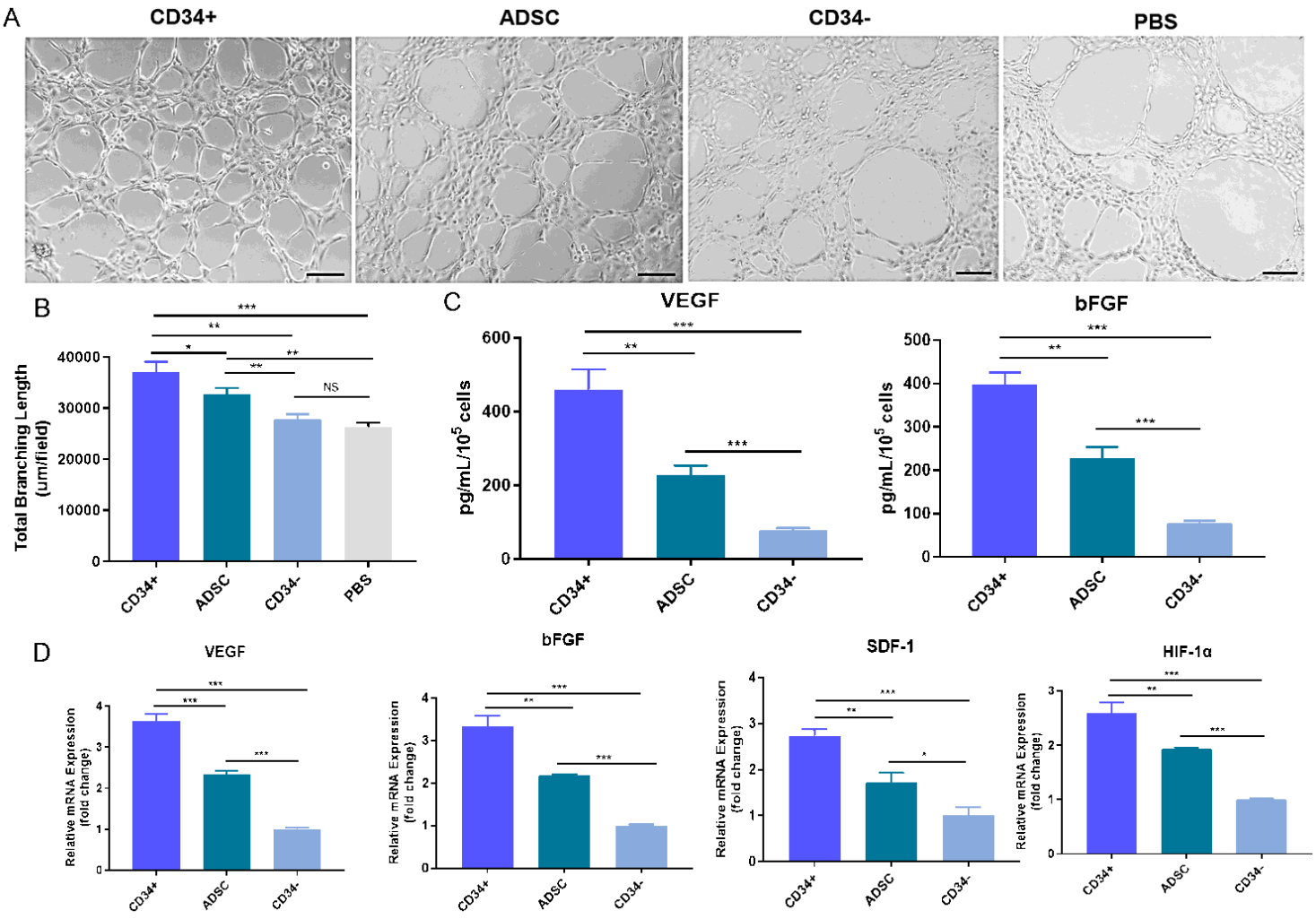

Figure 2

Adipose-derived CD34+ cells showed better proangiogenic capacity. (A-B) The total branch length of tubes formed in the CD34+ Group, ADSC Group and CD34- Group. (C) The secretion of proangiogenic-related factors in the CD34+ Group, ADSC Group and CD34- Group. (D) The expression of proangiogenic genes in the CD34+ Group, ADSC Group and CD34- Group. Data were analyzed using one-way ANOVA and Student's t test and expressed as the mean ± standard deviation of three separate experiments. *p<0.05; **p<0.01; ***p<0.001. Scar bar=100 μm.

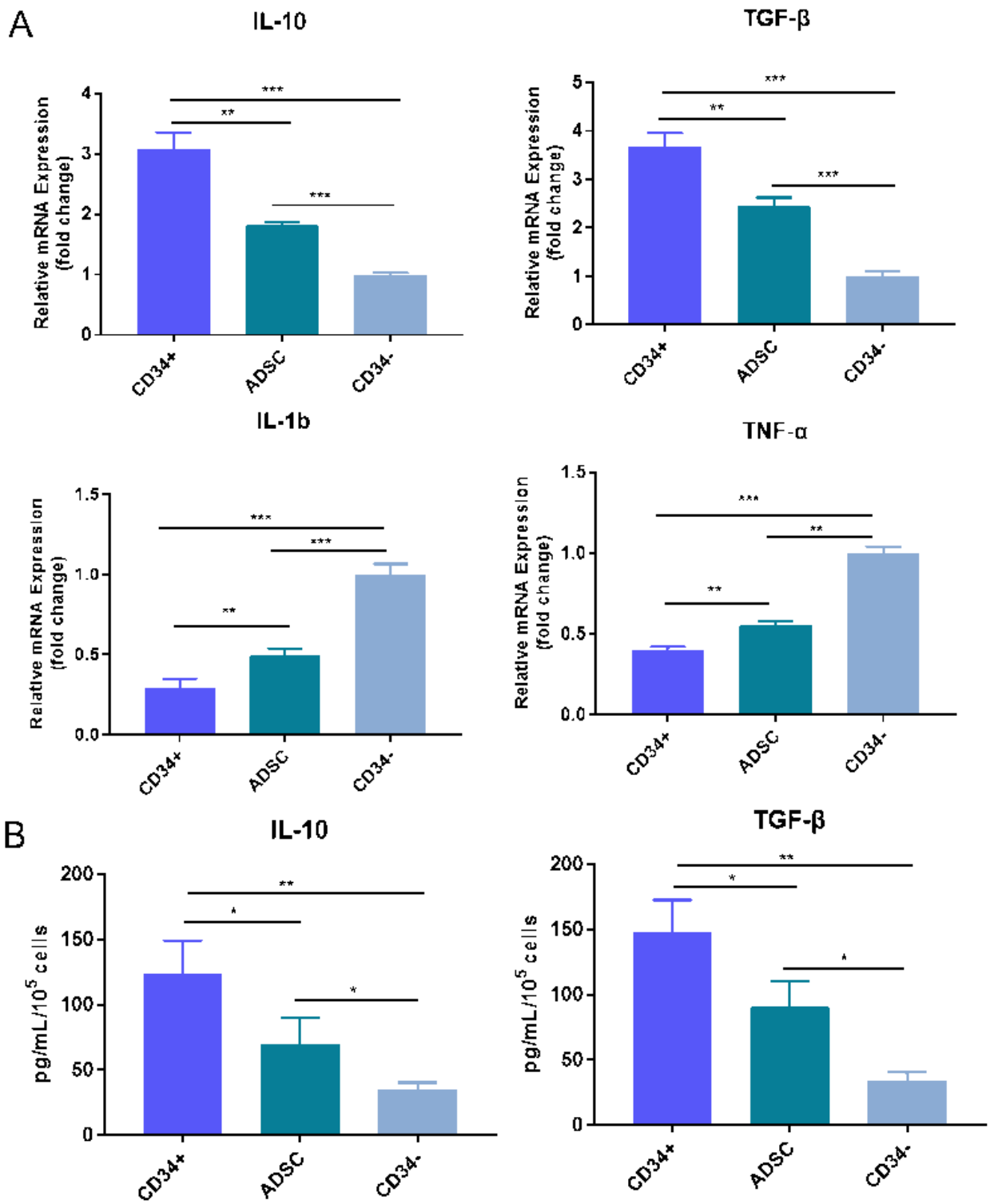

Figure 3

Adipose-derived CD34+ cells expressed higher anti-inflammatory factors and lower pro-inflammatory factors in vitro. (A) The relative mRNA expression (normalized to β-actin) of inflammation-related factors in the CD34+ Group, ADSC Group and CD34- Group. (B) The secretion of inflammation-related factors in the CD34+ Group, ADSC Group and CD34- Group. Data were analyzed using one-way ANOVA and

Student's t test and expressed as the mean ± standard deviation of three separate experiments. *p<0.05; **p<0.01; ***p<0.001.

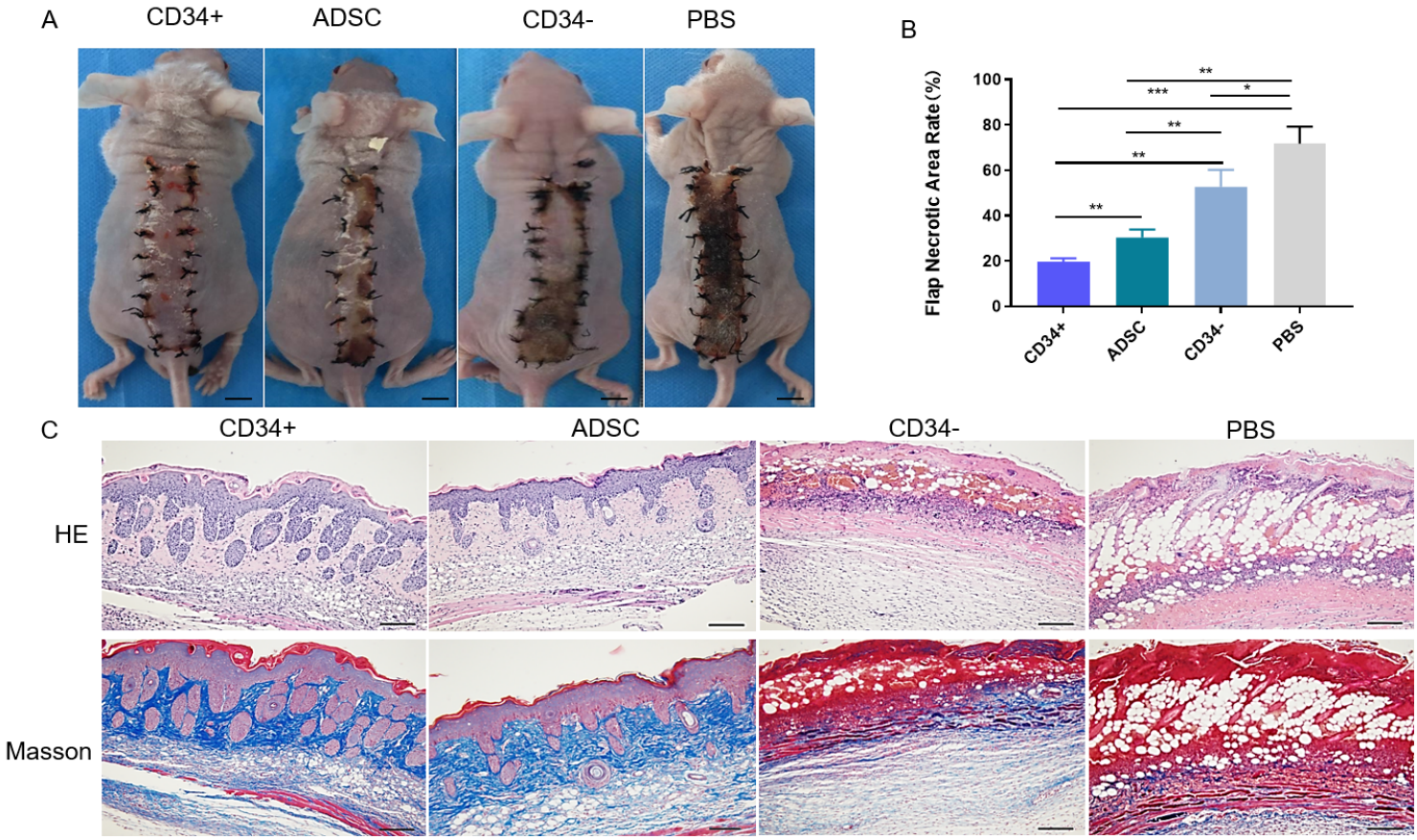

Figure 4

Adipose-derived CD34+ cells promote the survival of ischemic tissue. (A-B) The necrosis area rate of ischemic skin flaps in the CD34+ Group, ADSC Group and CD34- Group at 7 days after surgery. (C) H&E staining and Masson's trichrome staining in the CD34+ Group, ADSC Group and CD34- Group. Data were analyzed using one-way ANOVA and Student's t test and expressed as the mean ± standard deviation of three separate experiments. *p<0.05; **p<0.01***p<0.001; Scar bar=50 μm.

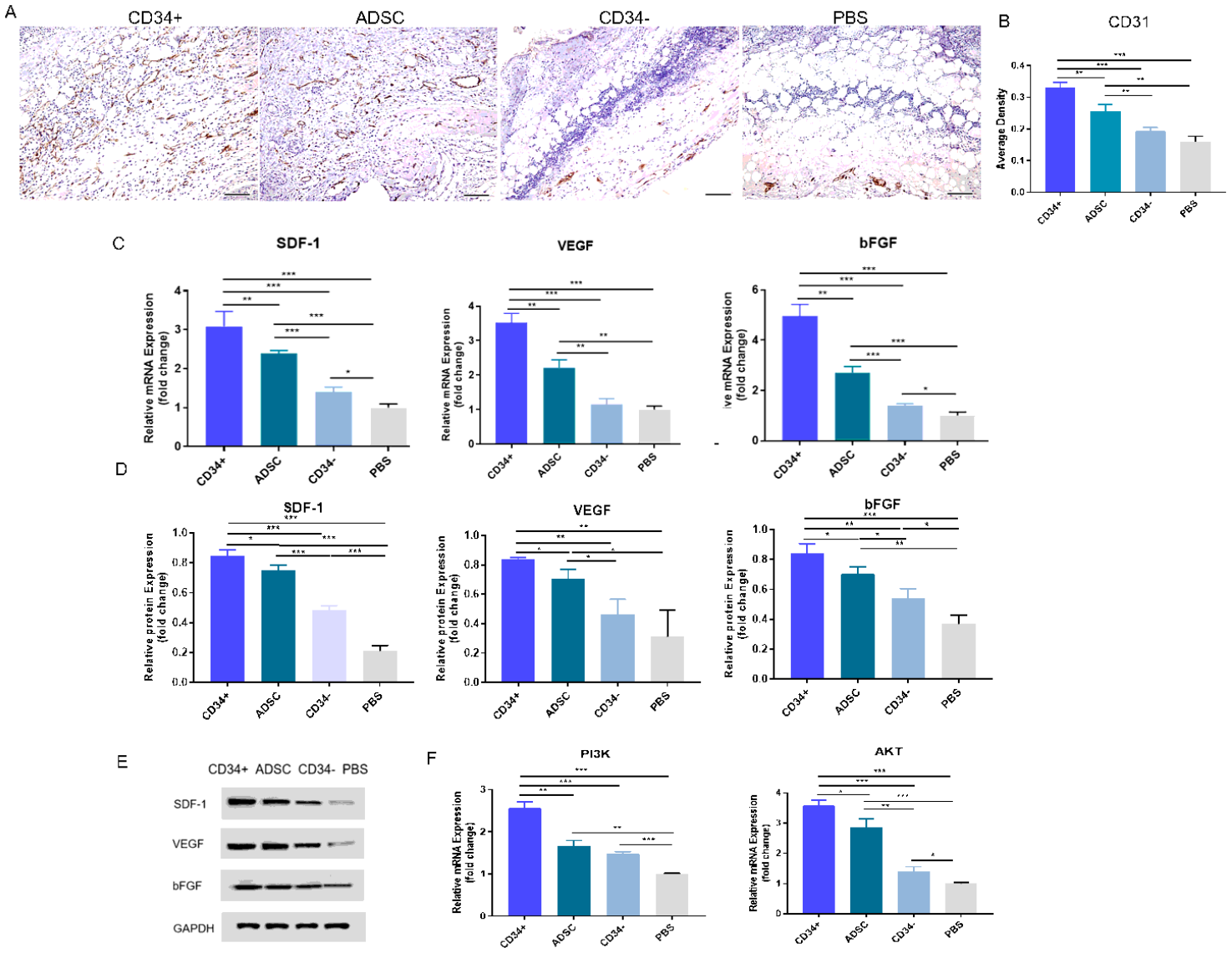

Figure 5

CD34+ cells showed superior proangiogenic potential. (A-B) The vessel density in the CD34+ Group, ADSC Group and CD34- Group by anti-CD31 histochemical staining of tissue sections collected on Day 7. (C-E) Relative mRNA expression (normalized to β-actin) of angiogenesis-related factors and proteins in the CD34+ Group, ADSC Group and CD34- Group. (F) Relative mRNA expression (normalized to β-actin) of PI3K and AKT in the CD34+ Group, ADSC Group and CD34- Group. Data were analyzed using one-way ANOVA and Student's t test and expressed as the mean ± standard deviation of three separate experiments. *p<0.05; **p<0.01；***p<0.001. Scar bar=100 μm.

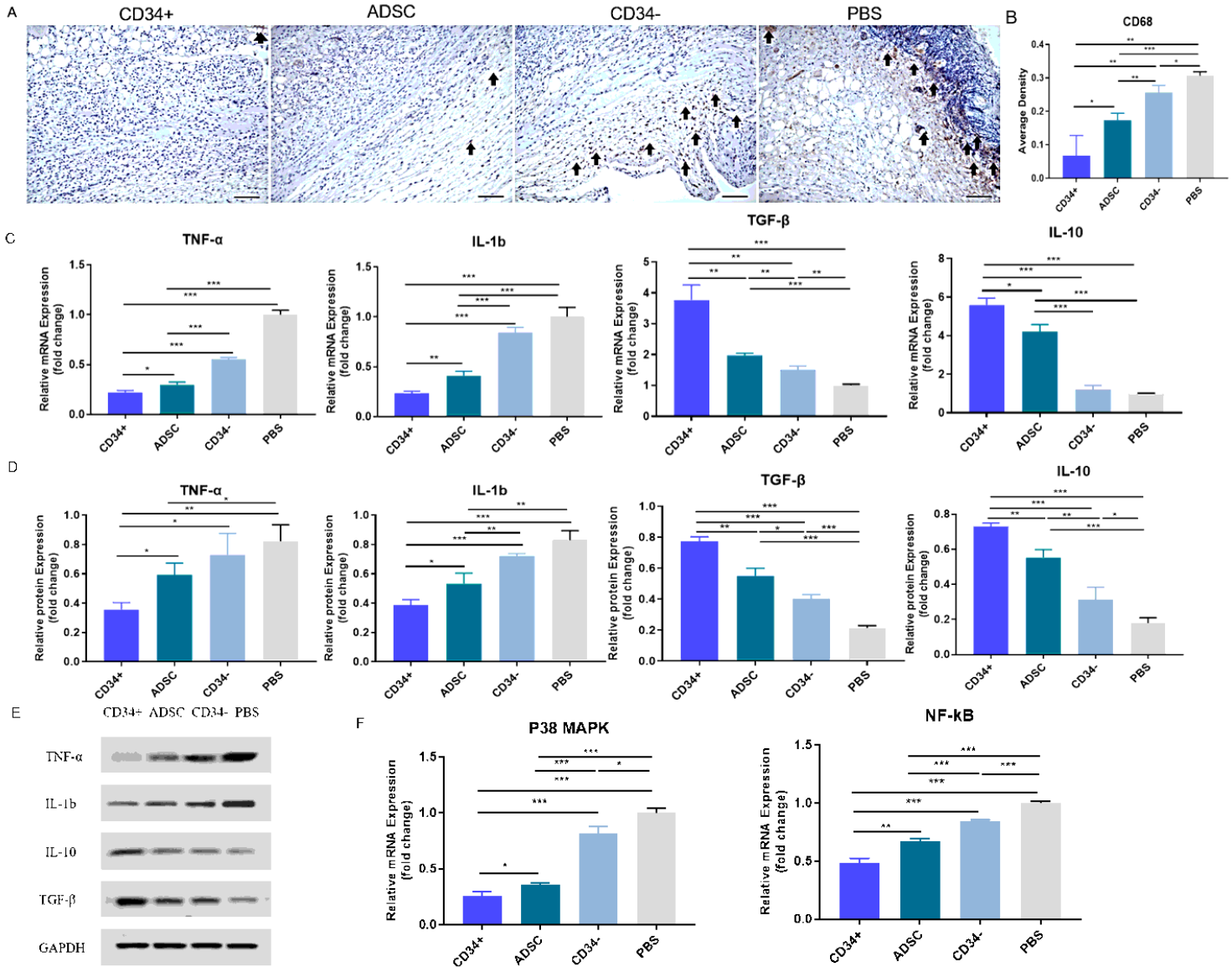

Figure 6

CD34+ cells suppressed immune cell infiltration. (A-B) Immune cell infiltration in the CD34+ Group, ADSC Group and CD34- Group by anti-CD68 immunohistochemical staining (CD68+ cells marked with black arrow). (C-E) Relative mRNA expression (normalized to β-actin) and protein expression of inflammatory cytokines in the CD34+ Group, ADSC Group and CD34- Group. (F) Relative mRNA expression (normalized to β-actin) of P38 MAPK and NF-kB in the CD34+ Group, ADSC Group and CD34- Group. Data were analyzed using one-way ANOVA and Student's t test and expressed as the mean ± standard deviation of three separate experiments. *p<0.05; **p<0.01；***p<0.001. Scar bar=100 μm.