

CXCL12⁺ Adipose Stem Cells Enhance Fat Graft Vascularization by Recruiting Endothelial Progenitors

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Research Article

Keywords: autologous fat grafting, adipose-derived stem cells (ADSCs), CXCL12, SDF-1 α , neovascularization

Posted Date: October 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10990461/v1>

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Additional Declarations: No competing interests reported.

Abstract

Autologous fat grafting is limited by unpredictable graft resorption, and early neovascularization is a critical determinant of graft survival. This study investigated whether co-transplantation of adipose-derived stem cells (ADSCs) overexpressing CXCL12 creates a chemotactic microenvironment that recruits endothelial progenitor cells (EPCs) and thereby promotes neovascularization. Human ADSCs were transduced to overexpress CXCL12. Fat grafts were mixed with CXCL12-overexpressing ADSCs (Group A), empty-vector-transduced ADSCs (Group B), unmodified ADSCs (Group C), or culture medium (Group D) and injected subcutaneously into nude mice. Circulating EPC counts and capillary density were assessed at multiple early time-points, and graft volume and histology on day 90. Group A showed sustained higher local SDF-1 α levels, with concomitant increases in circulating EPC counts and consistently higher capillary density at all early time-points compared with controls. On day 90, graft survival rates in Groups A–D were $71.8 \pm 1.7\%$, $55.8 \pm 1.5\%$, $56.1 \pm 0.8\%$, and $35.3 \pm 2.1\%$, respectively ($P < 0.001$). Group A grafts displayed well-formed adipose lobules, preserved interlobular septa, and reduced fat necrosis and fibrosis ($P < 0.01$). Collectively, local SDF-1 α overexpression by engineered ADSCs recruited circulating EPCs; the recruited EPCs, together with co-transplanted ADSCs, promoted neovascularization and improved long-term graft retention and histological quality.

Introduction

Autologous fat grafting is a promising technique for the repair, reconstruction, and augmentation of soft tissues. It is widely utilized due to its biocompatibility, natural aesthetic outcomes, readily available source material, and low donor-site morbidity (1, 2). However, its effectiveness in clinical application is hindered by the low survival rate and unpredictability of the grafted fat. The primary causes include inadequate early vascularization, graft resorption, and cyst formation resulting from ischemic necrosis (3, 4). Therefore, enhancing early angiogenesis within the grafted adipose tissue represents a key strategy to address this central issue.

Ongoing efforts to improve graft survival have explored various approaches, among which stem cell-based therapies show considerable promise. Adipose-derived stem cells (ADSCs) are adult multipotent mesenchymal stem cells that express characteristic surface markers such as CD105, CD90, CD44, and CD29, and possess multilineage differentiation potential (5, 6). They are known to secrete a variety of growth factors, cytokines, and extracellular matrix components, including VEGF, FGF-2, and collagens I–VI (7). ADSCs have been used in the studies of regenerative therapies (8) and cell-assisted lipotransfer (CAL) technique (9), which has been proved to improve the survival of graft and enhance vascularization. Endothelial progenitor cells (EPCs), another stem cell population derived from bone marrow and detectable in peripheral blood, express markers including CD133, CD34, and VEGFR-2 (10). Regarded as precursors to vascular endothelial cells, EPCs have been demonstrated to promote neovascularization (11) and contribute to both physiological and pathological postnatal vascular growth (12, 13). Previous studies support the pro-angiogenic potential of EPCs (14–16), and co-transplantation of mesenchymal stem cells (MSCs) with EPCs has been shown to enhance angiogenesis and osteogenesis (17, 18). Thus,

combining ADSCs with EPCs could represent a more effective strategy to promote angiogenesis in fat grafts.

However, the direct use of EPCs is constrained by challenges in isolation and expansion. Moreover, *in vitro* expansion of EPCs leads to diminished migratory capacity and partial loss of angiogenic function (18, 19). Given these limitations, direct co-transplantation is not ideal. As an alternative, stromal cell-derived factor1 α (SDF-1 α , also known as CXCL12) has been identified as a key chemokine that mediates EPC migration (20). SDF-1 α binds and activates two G protein-coupled receptors, CXCR4 and CXCR7, activating multiple downstream signaling pathways that regulate various biological processes in EPCs (21, 22). Previous evidence has indicated that SDF-1 α can promote re-endothelialization after vascular injury via SDF-1 α /CXCR4 axis (23). Locally delivered SDF-1 α promotes neovascularization in ischemic tissues by recruiting EPCs (24, 25).

Based on these findings, we hypothesize that transfection of ADSCs with CXCL12 to overexpress SDF-1 α can mobilize bone marrow-derived EPCs into the peripheral circulation and promote their homing to the fat graft site. This approach may mimic the beneficial effects of ADSC-EPC co-transplantation while circumventing the limitations associated with exogenous EPC use. We further aim to investigate whether this strategy can accelerate early angiogenesis within the graft and improve the survival rate of transplanted adipose tissue.

Materials and Methods

Sources of Human Fat Tissue

The ADSCs were obtained from a 23-year-old female donor undergoing abdominal liposuction. Meanwhile, fat tissue from a 27-year-old female patient who underwent the same procedure was harvested for transplantation. Both participants were in good health. This study was approved by Shanghai General Hospital Institutional Review Board (Approval Number: [2024]-070) and were conducted in accordance with the Declaration of Helsinki. Human participants were prospectively recruited from November 15, 2024, to November 21, 2024. Firstly, explain to each donor the types of fat tissue donation, the risks of the surgical procedure, post-operative precautions, complications and preventive measures, clearly state the compensation for donation, inform that they can withdraw voluntarily at any time; verify the donor's valid identification documents, inform of the medical assessment results, confirm that the donation does not impair their normal physiological functions; the donor voluntarily signs the "Informed Consent Form for Living Organ/Tissue Donation", clearly stating that only fat tissue will be donated; the informed consent document is submitted to the ethics committee of the medical institution for human organ transplantation for review. After being approved by more than two-thirds of the committee members, the acquisition of fat tissue can be carried out. The consent process was documented with signed consent forms, which were retained in accordance with institutional regulations.

Isolation and Cultivation of ADSCs

The procedure of isolated the ADSCs form the fatty portion of the liposuction aspirates according to the method reported by Yang X F (26), with some modification. Briefly, the fat tissue pieces were digested with 0.1% collagenase (sigma, America), then shaken in the oscillator for 50 min at 37°C in a 50 ml centrifuge tube. Then the collagenase used to digest the fat tissue was neutralized by equal volume of Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum, after which the cell suspension was centrifuged at 1400 rpm (300 g) for 5 minutes. The ADSCs were cultured in the DMEM at a concentration of 6000 cells/cm² per tissue culture plate and maintained at 37°C in 5% CO₂. The medium was replaced every 3 days, and the nonadherent cells were discarded. Cells were passaged at a ratio of 1:3 upon reaching 80–90% confluence. Cells cultured for three passages were used in further study.

Identification of ADSCs

The ADSCs were detached by treatment with 0.05% trypsin-edetic acid (Invitrogen, Carlsbad, California) and subsequently suspended in PBS supplemented with 0.5% bovine serum albumin, at a density of 5×10⁵ cells per tube. The cells were incubated with either isotype controls or antigen-specific antibodies in PBS at 4°C for 30 minutes. The antibodies used included FITC-conjugated anti-human mouse antibodies targeting anti-CD29, anti-CD31, anti-CD34, anti-CD44, anti-CD45d, anti-CD105, anti-HLA-DR (all from BD Biosciences, San Jose, California) and anti-CD133 (Miltenyi, Germany). After staining, the cells were washed three times with PBS and analyzed by flow cytometry (BD Biosciences). Additionally, the third passage cells were induced into osteogenic and adipogenic differentiation to assess their differentiation capacities.

Lentivirus Generate and Transfection

The gene sequence of CXCL12 was obtained from the Genbank database. The primers were designed using Primer 5 software and synthesized by Hanhbio Company. Lentiviral vectors were generated using the lentivirus packaging system (Tronolab), which is a four-plasmid system consisting of pRSV-Rev, pMDlg-pRRE, pMD2G, and the Transfer Vector. The lentiviral expression plasmid can express green fluorescent protein (GFP), whereas pRSV-Rev, pMDlg-pRRE, and pMD2G contain the necessary components for virus packaging. The titer of lentivirus was determined using attenuation method and flow cytometry, and found to be 1.4 x 10⁹ TU/ml. The best MOI used to infect the ADSCs was determined to be 50, and the transfected cells were visible under green fluorescence. Following puromycin screening, the cells were amplified and growth curve was measured.

Determination of SDF-1α Expression of ADSCs in vitro

The secretion of the SDF-1α protein by three types of ADSCs, namely CXCL12-transfected ADSCs (CXCL12⁺ADSCs), empty vector-transfected ADSCs (eVec⁺ADSCs), and un-transfected ADSCs (un-trans

ADSCs), was evaluated using the Western blot and elisa. All the three types of ADSCs being evaluated are third-generation cells. The cell culture medium was collected 2, 4, 7, 14 days after gene transfection.

Protein was extracted and quantified using a total protein extraction kit and a BCA protein assay kit (cat. nos. P0013G and P0010, both from Beyotime, Beijing, China) following the manufacturer's instructions. Proteins were separated using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE, cat. no. PG113, EpiZyme, Shanghai, China) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA) The membranes were blocked at room temperature for 30 min with protein-free rapid blocking buffer (cat. no. PS108P, EpiZyme). Primary antibodies were added, and the membranes were incubated overnight at 4°C with gentle shaking. The primary antibodies used were anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:20000, cat. no. 10494-1-AP, Proteintech, China), anti-CXCL12 (0.1 µg/ml, cat. no. ab9797, Abcam, UK). After washing the membranes three times with TBST, secondary antibodies (1:10000, Abcam, UK) were added for 2 h at room temperature. Western blot bands were visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences, USA), and band densities were quantified using ImageJ software, normalized to GAPDH expression, and compared with control group values.

Enzyme-linked immunosorbent assay (ELISA) The concentration of secreted CXCL12 in the ADSCs culture medium were measured with the DuoSet ELISA kit (cat. DY460, R&D Systems, USA) following the manufacturer's instructions. Briefly, 96-well plates were coated with CXCL12 antibody, and 100 µL of standards or samples were added to each well, followed by a 2-h incubation at room temperature. After washing each well three times, 100 µL of detection antibody was added, and the plates were incubated for another 2 h at room temperature. Following three additional washes, 100 µL of substrate solution was added and incubated for 20 min at room temperature in the dark. The absorbance was measured at 450 nm using a multifunctional microplate reader (BioTek Synergy, VT, USA).

Animal Model and Groups

All animal studies were approved by the ethics committee of Shanghai General Hospital (Approval Number: 2025AW054). All the surgeries were performed under pentobarbital sodium anesthesia, and every effort was made to alleviate the pain. The sample consisted of male BALB/c-nu mice, aged 6–8 weeks and weighing between 20-24g. 72 nude mice were randomly divided into 4 groups, with 18 mice in each group. Fat grafts were prepared using Coleman technology (27). The mice in the 4 groups were then injected subcutaneously on their backs with different fat tissue mixtures using 14-G needles. Group A, group B and group C received 5×10^6 CXCL12⁺ADSCs, eVec⁺ADSCs, un-trans ADSCs respectively, which were suspended in 0.2 ml of DMEM medium and uniformly mixed with 0.3 ml of adipose tissue (28). Finally, Group D was administered 0.2 ml of DMEM medium mixed with 0.3 ml of adipose tissue, representing the blank control group.

3 nude mice were randomly selected in each group at 2, 4, 7, 14, and 30 days after transplantation. To collect the venous blood, anticoagulant test tubes were used, and these were marked according to their

respective groups and time points. Afterward, all the mice were euthanized with carbon dioxide and then underwent a second cervical dislocation procedure, and their grafts were dissected from the subcutaneous area of their backs. Each graft was weighted using an electronic balance immediately, while its tissue volume was measured using the liquid overflow method.

EPC detection in peripheral blood

1mL venous blood were draw from each mouse. Mononuclear cells were separated using Ficoll gradient separation from the samples (29). The mononuclear cells were stained using FITC-labelled anti-CD34, PE-labelled KDR (both from BD Biosciences, San Jose, Calif), and APC-labelled anti-CD133 (AC133) antibodies (Miltenyi Biotech, Cologne, Germany). Finally, the sample cells were analyzed by a FACSCalibur flow cytometer, along with CellQuest Pro software.

Determination of SDF-1 α

Grafted fats from the 4 groups were harvested at 2,4,7,14, and 30 days, and then protein expressions of CXCL12/SDF-1 α of the grafted fat by using Western blot. Cell lysates were prepared using SDS lysis buffer. The BCA Protein Assay Kit (71285-M, ThermoFisher Scientific, Waltham, MA, USA) was used to quantify protein concentrations. For immunoprecipitation, cells were lysed with IP Lysis Buffer (20–188, ThermoFisher Scientific, Waltham, MA, USA) containing 1% cocktail of proteinase and phosphatase inhibitor and PMSF (S8830 and 329-98-6, ThermoFisher Scientific, Waltham, MA, USA). Equal amounts of protein lysate were incubated overnight at 4°C with antibodies and protein A/G magnetic beads (HY-K0202, MedChemExpress, Monmouth Junction, NJ, USA). Then the beads were washed at least three times with lysis buffer, and proteins were eluted with 1×loading buffer for WB. Whole cell lysates or immunoprecipitated proteins were resolved by SDS-PAGE. The proteins in the gel were transferred to PVDF membranes.

Determination of Neovascularization

Vascular endothelial cells of transplantation tissue paraffin sections were labeled using the goat anti-mouse CD34 antibody. Subsequently, in each of the 10 randomly selected and non-repeated fields with a 200× magnification under the light microscope, the number of microvascular was counted. Microvascular densities were measured using the method of tumor microvascular density, where the count consisted of stained microvascular, stained cell clusters as well as single-stained EPC. The counting was performed by two blinded reviewers.

Survival Rate and Histological Evaluation of Grafts

Three months post-transplantation, the volumes of the fat grafts were measured by the liquid overflow method, and the survival ratio was calculated using the formula: survival volume divided by pre-

transplant volume (0.3 ml). Thereafter, the fat grafts were fixed in 4% paraformaldehyde for 24 hours and embedded in paraffin for sectioning. Tissue sections from the center of the fat grafts were stained with hematoxylin-eosin (HE) for analysis. The tissue structure of the graft was meticulously observed under a microscope

Statistical Analysis

Data were expressed as mean $\pm$ SD and analyzed for repeated-measure analysis of variance (ANOVA) by SPSS18.9 (SPSS, Chicago, IL, USA). LSD test was used for pair-wise comparison between groups with the same variance. $P < 0.05$ was considered statistically significant.

Results

Identification of ADSCs

The identification of ADSCs involves two important aspects: the expression of cell surface markers and the verification of their multi-directional differentiation potential. Before transfection, the expression of surface markers on ADSCs was verified by flow cytometry. Additionally, their multi-lineage differentiation potential was evaluated. Flow cytometry analysis revealed that the cells were positive for mesenchymal stem cell-related molecules CD105 ($> 90\%$) and CD44 ($> 80\%$), while markers for hematopoietic or endothelial cells, such as CD31, CD34, HLA-DR, and CD45, were negative (Fig. 1A). Alizarin Red staining for osteogenic differentiation showed the formation of calcium nodules in ADSCs after 14 days of induction (Fig. 1B); Oil Red O staining results indicated the formation of lipid droplets in ADSCs after 10 days of induction (Fig. 1C). The results suggest that these cells exhibit strong osteogenic and adipogenic differentiation capabilities.

SDF-1 α Expression of ADSCs In Vitro

The SDF-1 α protein secreted by ADSCs was detected by Western blot. The results showed that compared with the control group and the empty vector group, the expression of SDF-1 α in CXCL12 $^{+}$ ADSCs was significantly increased, and the difference was statistically significant ($P < 0.001$, Fig. 2A-B). Additionally, the ELISA experiment also confirmed that the culture supernatant of CXCL12-positive ADSCs contained a higher concentration of SDF-1 (Fig. 2C).

The Evaluate of Vascular Formation in Grafts

The content of endothelial progenitor cells (EPCs) in the peripheral circulation was evaluated by flow cytometry on the 2nd, 4th, 7th, 14th and 30th days after fat transplantation (Fig. 3A). The results showed that there were differences in the number of EPCs in the peripheral blood of the four groups. The number of EPCs in the CXCL12 $^{+}$ ADSCs group was higher than that in the other groups at all time points, and the number in the DMEM group was the lowest. Statistical analysis showed that the differences in EPCs in the peripheral blood of the four groups were statistically significant (Fig. 3B). Subsequently, the

expression of SDF-1 α protein in the transplanted tissues was detected. At all time points, the expression of SDF-1 α protein in the CXCL12⁺ADSCs group was higher than that in the other three groups, and it showed a time-dependent manner (Fig. 3C-D).

Determination of Neovascularization by Measuring Microvascular Density

Microvascular density is an important indicator of neovascularization. Therefore, we selected ten regions from the central area of the transplanted tissue to assess the microvascular density. At all time points, the microvascular density of the grafts in Group A (CXCL12⁺ ADSCs) was significantly higher than that of the other three groups. At the first two time points, the microvascular density of the grafts in Group B (eVec⁺ ADSCs) and Group C (ADSCs) was also well maintained and comparable. On the other hand, the number of countable microvessels in the transplanted body of Group D (DMEM) was the lowest. Through the use of Fisher's Least Significant Difference (LSD) test, these differences were proven to be statistically significant. However, at the last three time points, there was no significant difference in microvascular density among Groups B, C, and D (Figs. 4A-C).

The Survival Rate of Fat Grafts and Histological Assessment

The volumes of these 4 groups of grafts were measured using the liquid overflow method. Then, the survival rate was calculated according to the formula: survival volume divided by the previous volume (0.3 ml). The survival rates of Groups A, B, C, and D were $71.8 \pm 1.7\%$, $55.8 \pm 1.5\%$, $56.1 \pm 0.8\%$, and $35.3 \pm 2.1\%$ ($n = 4$), respectively. It was found that the differences between Group A and Groups B, C, and D were significant ($P < 0.001$). However, there was no statistically significant difference between Groups B and C (Fig. 5A-B).

Further histological assessment of the fat grafts revealed that the transplantation samples of the blank control group (Group D) showed excessive fibrosis and fat necrosis, making it difficult to identify the normal fat lobule structure. Similarly, the transplantation samples of Groups B and C, which were mixed with untransfected and empty viral vector-transfected ADSCs, also exhibited partial fibrosis and fat necrosis, but showed more normal fat lobule structures. In contrast, the transplantation samples of Group A, which were mixed with CXCL12⁺-transfected ADSCs, contained adipose tissue with mature lobule structures, and the lobule septa remained intact. Moreover, the degree of fat necrosis and fibrosis in these transplantation samples was significantly lower (Fig. 5C-D).

Discussion

Autologous fat transplantation is a promising technique utilized for repair and reconstruction of soft tissue defects. Its clinical application is hindered by the uncertain survival rate of the grafts. Ischemic

necrosis, liquefaction, and gradual absorption of grafted fat occur due to inadequate and delayed vascular reconstruction, which ultimately leads to a survival rate that varied between 20% and 80% (30, 31). Attaining adequate vascularization of the grafted fat during the early stages is crucial for improving its survival rate (3). Various techniques have been utilized to stimulate angiogenesis and improve the survival rate of grafted fat, such as cytokines-assisted approaches (32, 33), cell-assisted techniques (9), SVF (34), PRP (35), and other approaches (36). However, these techniques have some limitations and shortcomings. Multiple cytokines and related cells are needed in the angiogenesis process, and a single regulation approach may not be sufficient to stimulate angiogenesis (37).

Stem cell therapy is a method that uses the self-renewal, differentiation replacement and secretion of regenerative cytokines to renew and replace dysfunctional tissues or organs (38). Despite the numerous candidates for stem cells, ADSCs have emerged as the most promising stem cell population for their acquired availability, wide range of sources, and low donor-site morbidity. In recent years, the application of ADSCs in regenerative medicine is mainly due to their multilineage differentiation potential and paracrine activity (39, 40). In our experiment, the transplants were mixed with un-transfected or empty virus vector-transfected ADSCs (Group C and B), and SDF-1 α was higher than Group D (DMEM) at each set time point, which suggests that ADSCs can secrete SDF-1 α under specific circumstances. Meantime, Group B and C showed an improvement in the microvascular density and residual fat mass, which is consistent with the CAL results. The specific reason for this performance can be attributed to the proangiogenic potential and anti-inflammatory effects of ADSCs. Firstly, the ADSCs can differentiate into vascular smooth muscle cells (41), endothelial cells (42, 43) and pericyte-like cells (44, 45) to participate in angiogenesis (although this differentiation requires certain inducing conditions, it cannot be completely denied that there are no corresponding conditions when adipose tissue is transplanted in vivo). Secondly, ADSCs can secrete vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF- β), platelet derived growth factor (PDGF), angiogenin (ANG), stromal cell derived factor-1/chemokine (CXC motif) ligand 12 (SDF-1/CXCL12) and other angiogenic cytokines, which can synergistically promote angiogenic process (46, 47). Thirdly, the ADSCs can modulate inflammatory and oxidative responses via crosstalk between the Nrf2 and TLR4 pathways (48).

Cell co-implantation represents an advanced model of cell-based therapy (49). A variety of cells or stem cells are transplanted together to harness the direct and indirect effects of multiple cell types to expedite angiogenesis and enhance graft survival (50). The communication and coordination between co-implantation cells can augment their angiogenic potential, ultimately yielding superior outcomes compared to stem cell alone (51, 52). However, due to in vitro expansion resulting in reduced EPCs migration capacity and a partial loss of their vasculogenic ability (52, 53). Our results showed that elevated levels of SDF-1 α expression in Group A were observed at each set time point, the highest number of EPCs were detected in the peripheral blood of Group A, and the number of labeled endothelial cells in the graft sections was determined. These results indicated that SDF-1 α expression could be upregulated by ADSCs transfected CXCL12, and the upregulated SDF-1 α could mobilize EPCs into the peripheral blood circulation and migrate to the site of adipose graft, which is feasible to recruit EPCs to the graft site and work with ADSCs to achieve similar effects as co-transplantation of the two cells. SDF-

1 α can not only mobilize chemotactic EPCs via the SDF-1 α /CXCR4 pathway to promote angiogenesis (25), but also can promote angiogenesis via induce migration and proliferation of tissue-specific progenitors or stem cells (54, 55). SDF-1 α also possess the capability to recruit and retain mural/accessory cells (pericytes and vascular smooth muscle cells), these cells play a crucial role in promoting the maturation and stabilization of newly formed vascular (56, 57). SDF-1 α /CXCR4 pathway also participate in vascular remodeling by recruiting smooth muscle progenitor cells (58). In addition, the SDF-1 α /CXCR7 pathway can enhance the survival of EPCs and encourage the secretion of angiogenic growth factors by these cells (59, 60).

In our study, the way to chemotactic EPCs via CXCL12⁺ADSCs was different from traditional co-transplantation of ADSCs and EPCs. These differences are primarily manifested in temporal and spatial aspects. In terms of time, unlike the co-transplantation of both cells, the way to chemotactic EPCs involves initially transplanting CXCL12 + ADSCs and subsequently obtaining EPCs through CXCL12 + ADSCs chemotaxis, with a sequential involvement of these two cell types in the experimental process. In terms of spatial distribution, unlike the direct contact observed in co-transplanted cells, ADSCs are uniformly dispersed within the transplanted fat. Conversely, in the EPC chemotaxis method chemotactic EPCs are theoretically localized only at the periphery of capillaries surrounding the graft. However, the results showed in our experiment, compared with Group B and Group C, Group A (CXCL12⁺ADSCs) had further improvement in both microvascular density and residual fat volume, which illustrated the way to chemotactic EPCs could also improve the vascularization and survival of fat grafts. Despite the temporal and spatial disparities existing between the chemotactic EPCs method and the co-transplantation of the two cells, these variations do not carry significant drawbacks. In fact, they can even be advantageous in stimulating vascularization and enhancing graft survival. Firstly, the chemotactic EPCs approach eliminates the *in vitro* expansion process, thereby avoiding the reduced migratory capacity of EPCs and the maturation of the cells into endothelial cells that result in partial loss of angiogenesis (52). Secondly, during the initial stages of the EPCs approach, although direct contact mediated mutual regulation between EPCs and ADSCs may be lacking, these two cell types possess the ability to influence each other through paracrine signals (61–63). The enhanced vascularization and adipose viability observed in our study could be attributed to the synergistic action of ADSCs and EPCs. ADSCs possess differentiation potential, allowing them to transform into specific cells that actively contribute to angiogenesis within a distinct microenvironment. Moreover, ADSCs promote the angiogenesis via a paracrine an array of cytokines.

EPCs are responsible for turning on the “angiogenic switch” in neo angiogenesis (64, 65). EPCs consist of at least two subtypes, the “early EPCs” and “late EPCs” with different morphologies and growth patterns. Late EPCs exert structural functions through vascular integration and early EPCs provide instructive functions through paracrine release of multiple pro-angiogenic cytokines, such as VEGF, insulin-like growth factor I (IGF-I), SDF-1, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 α , and PDGF (66–68). Although the exact mechanism is still unclear, we

speculated that the complex synergy effect of multiple cytokines, cells, and pathways that promote vascularization of transplanted fat.

We acknowledged that this study still existed several limitations. Firstly, the small number of specimens at each time point restricts the generalizability of the findings. Furthermore, the specific mechanism that promotes graft revascularization remains unclear. It is crucial to highlight that, at present, cell and gene therapies are not accessible for clinical application.

Conclusion

We demonstrated that upregulating the expression of CXCL12 in ADSCs effectively attracts EPCs to the transplantation site. The synergistic effect of chemotactic EPCs and transplanted ADSCs promotes early vascularization and enhances the survival rate of transplanted fat, which is like the effect of indirect contact co-transplantation of EPCs and ADSCs. This methodology circumvents the disadvantages associated with in vitro culturing of EPCs and presents a promising vascularization strategy.

Declarations

Competing interests

The authors declare no competing interests.

Ethical statement and consent to participate

The animal study protocol was approved by the ethics committee of Shanghai General Hospital (Approval Number: 2025AW054) and strictly adhered to the ARRIVE 2.0 guidelines throughout. The human specimen study received approval from Shanghai General Hospital Institutional Review Board (Approval Number: [2024]-070). None of the transplant donors was from a vulnerable population and all donors or next of kin provided written informed consent that was freely given. The entire research process strictly followed the Declaration of Helsinki.

Consent for publication

Not applicable.

Funding

Not applicable.

Author Contribution

Writing—original draft preparation, methodology and data curation: M.W. and D.L.; Conceptualization, resources, project administration: J.Z.; formal analysis, validation and writing—review and editing: A.L. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

Not applicable.

Data availability

The data underpinning the present findings are fully disclosed within the manuscript, and additional raw data in alternative formats may be procured from the corresponding author upon substantiated academic request.

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Figures

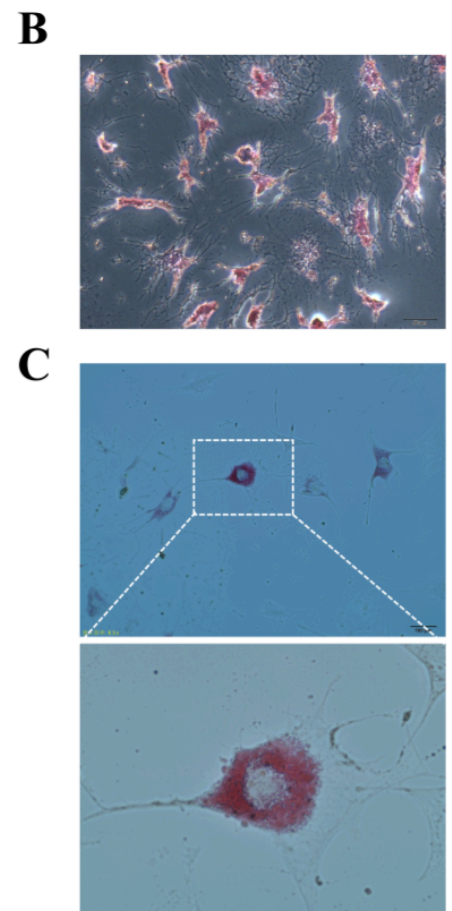
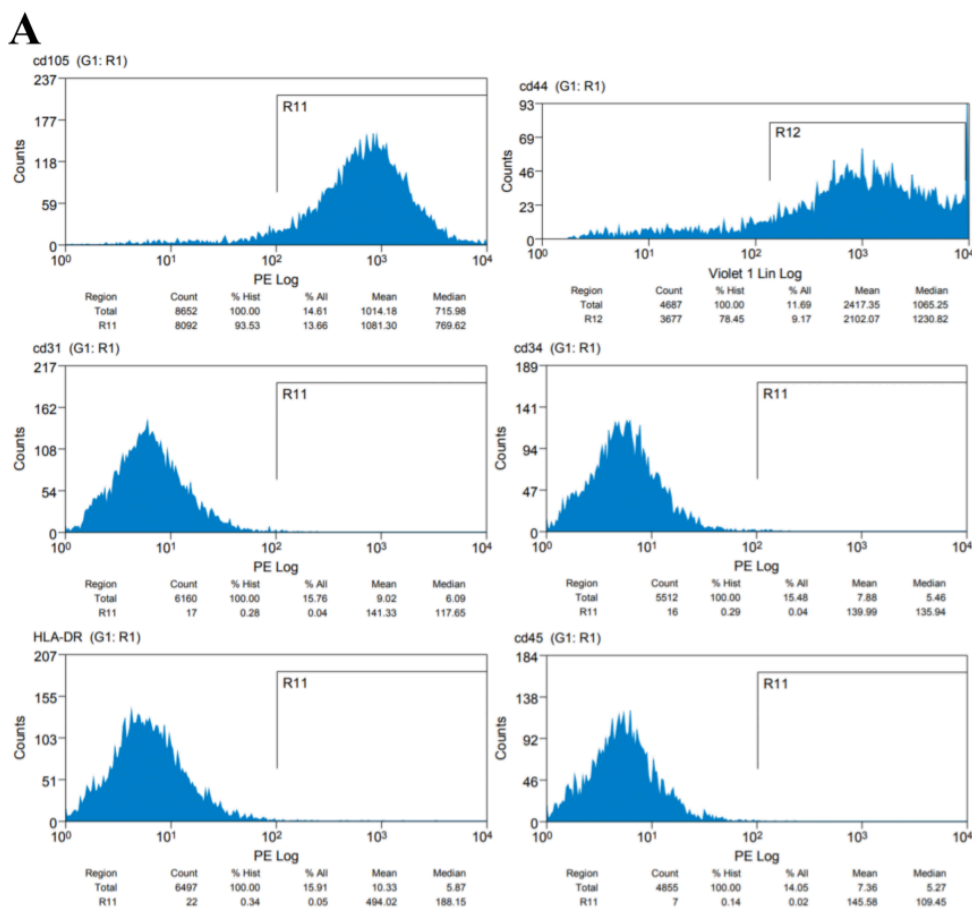


Figure 1

Identification of ADSCs. (A) Flow cytometric analysis revealed that the cells positively expressed CD105 (>90%), and CD44 (>80%), while CD31, CD34, HLA-DR, and CD45 were all negatively expressed. (B) Detection of the multipotent capacity of ADSCs to induce osteogenic differentiation by Alizarin Red staining. (C) Detection of the multipotent capacity of ADSCs to induce adipogenic differentiation by Oil Red O staining.

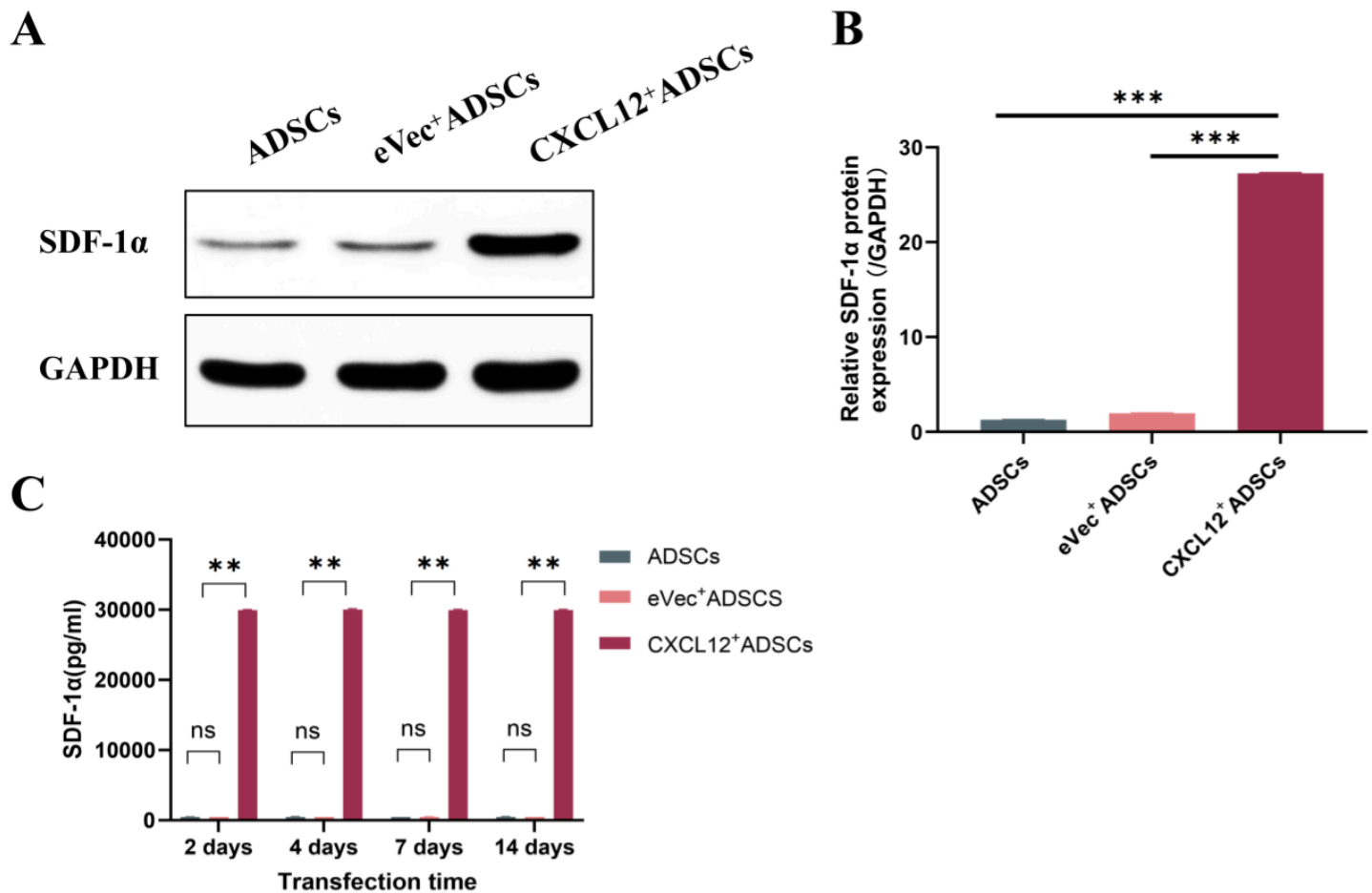


Figure 2

The detection of SDF-1α expression of ADSCs *in vitro*. **A** Western blot analysis of SDF-1α protein expression in ADSCs, eVec+ADSCs and CXCL12+ADSCs were measured by Western blot. **B** Western blot quantitative analysis of SDF-1α expression level. **C** ELISA detection of SDF-1α secretion in the culture supernatant of ADSCs. * P < 0.05, ** P < 0.01, *** P < 0.001, ns P > 0.05.

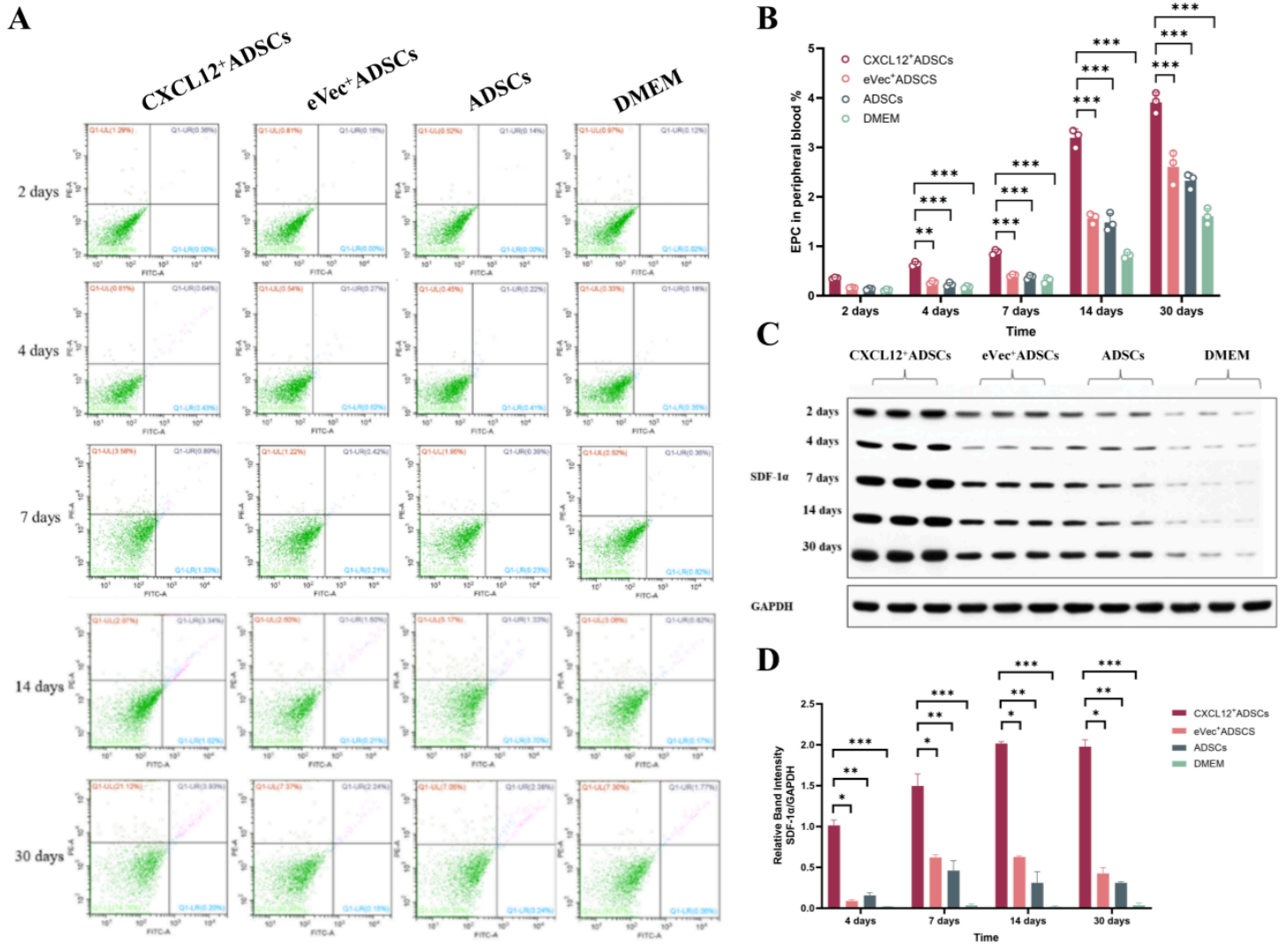


Figure 3

Evaluation of the function of the grafts. A-B.Peripheral blood EPC were measured by flow cytometry at the 2, 4, 7, 14, 30 days after fat transplantation (EPC percentage of peripheral blood mononuclear). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **C-D.**SDF-1α protein expression in fat tissue at different time after transplantation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

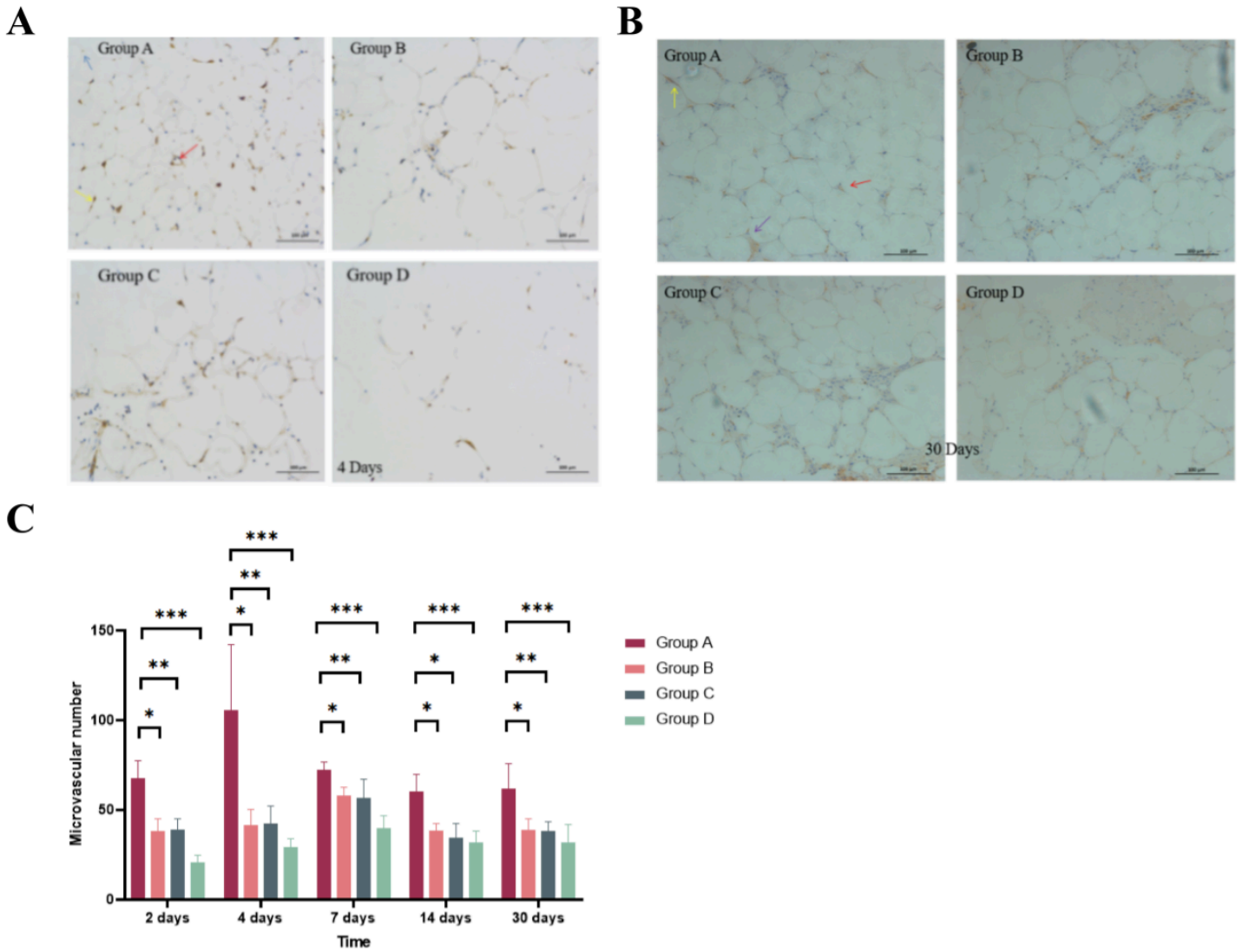


Figure 4

Determination of Neovascularization by Measuring Microvascular Density. **A.** The fourth days of immunohistochemical (IHC) staining of the blood vessels. **B.** The IHC staining of blood vessels for thirtieth days. **C.** Statistical analysis of microvascular density by LSD test at the 2, 4, 7, 14, 30 days after fat transplantation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

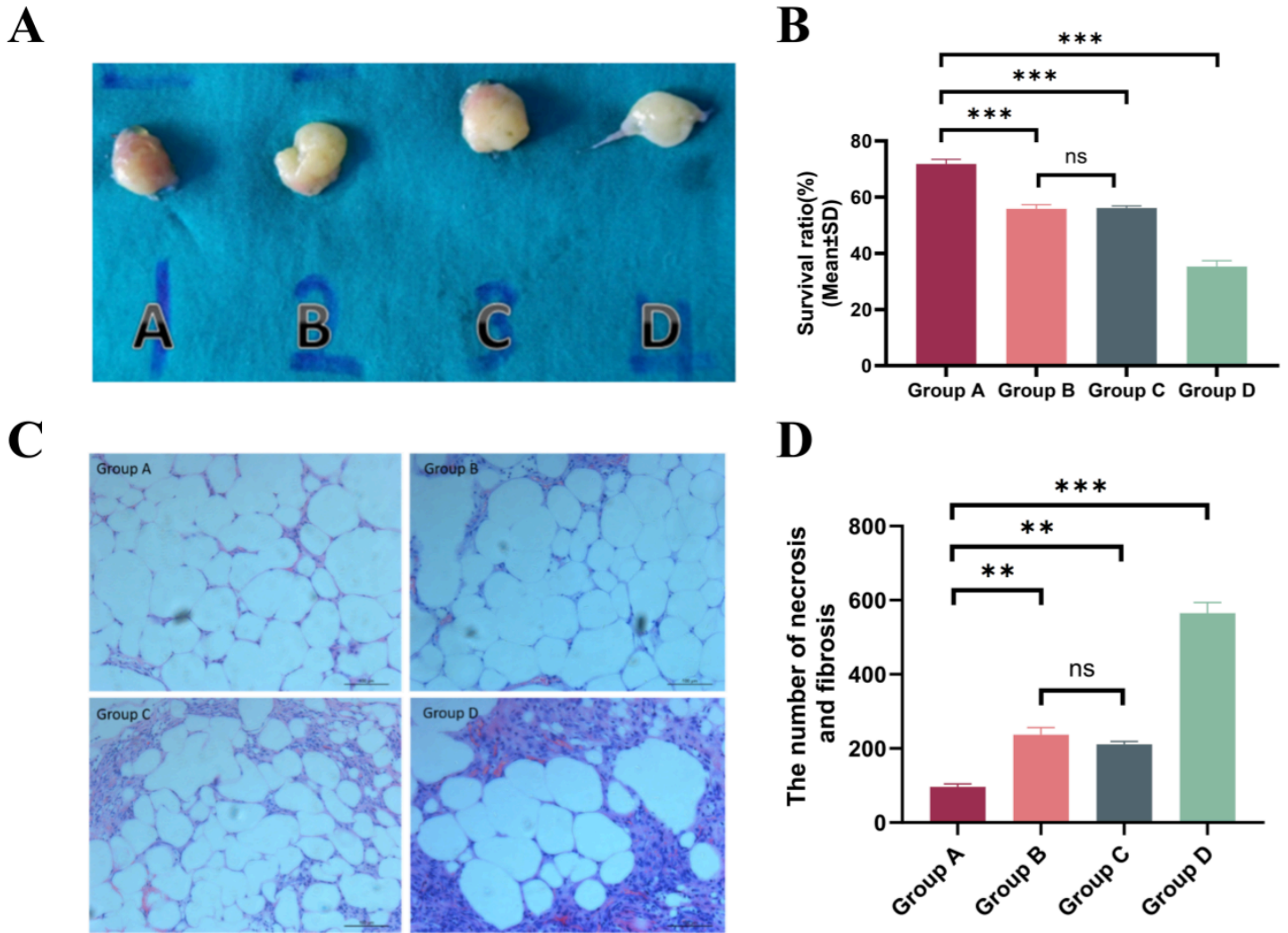


Figure 5

The Survival Rate of Fat Grafts and Histological Assessment. **A.** Survival of free fat grafts of Group A, B, C, D after 90 days. **B.** The survival ratios of the grafts were calculated by using the formula survival volume/previous volume (0.3 ml). The bars shown the average survival rate of the four Group A, B, C and D, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P > 0.05$. **C-D.** The “point counting method” was used to measure the degree of fibrosis and liquefaction necrosis of adipose tissue sections after 90 days. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P > 0.05$.