Human Adipose-Derived Stem Cells Can Optimize the Filling Material in Rats

Siwei Qu
Chinese Academy of Medical Sciences and Peking Union Medical College
Ning Ma
Chinese Academy of Medical Sciences and Peking Union Medical College
Weixin Wang
Chinese Academy of Medical Sciences and Peking Union Medical College
Sen Chen
Chinese Academy of Medical Sciences and Peking Union Medical College
Qi Wu
Chinese Academy of Medical Sciences and Peking Union Medical College
Yangqun Li
Chinese Academy of Medical Sciences and Peking Union Medical College
Zhe Yang (yangzhe@psh.pumc.edu.cn)
Chinese Academy of Medical Sciences and Peking Union Medical College

Research Article

Keywords: Human adipose-derived stem cells, Porcine acellular dermal matrix, filling material

Posted Date: July 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1797189/v1

Abstract

Introduction: Human adipose-derived stem cells have been identified as a promising candidate for cell-assisted therapy to improve graft survival. Human adipose-derived stem cells were added into filling materials.

Methods: The filling materials were prepared and divided into 6 groups: Fat particles with phosphate buffer saline or human adipose-derived stem cells; Acellular dermal matrix particles with phosphate buffer saline or human adipose-derived stem cells; Mixture of fat particles and acellular dermal matrix particles with phosphate buffer saline or human adipose-derived stem cells. The survival rate, vascular density and histological at 2, 6 and 12 weeks were investigated.

Results: Human adipose-derived stem cells significantly improved survival rate in each group at 6 and 12 weeks, and it significantly increased the vascular density in the fat particles and porcine acellular dermal matrix combined group and porcine acellular dermal matrix group at three time points, but human adipose-derived stem cells did not have a significant effect in the fat particles group.

Conclusion: Human adipose-derived stem cells as assisted cells added into filling material can improve survival rate and vascular density in rats.

Introduction

Filling injection is one of the minimally invasive methods for the treatment of tissue loss of volume, contours and static wrinkle. The popularity and acceptance of filling injection have risen steadily. A 297% increase in the use of soft tissue fillers between 1997 and 2008 was reported by the American Society of Aesthetic Plastic Surgery. An more than 44% increase between 2011 and 2016(1). Injection filling materials can be divided into synthetic materials and biological materials. The biological materials include hyaluronic acid, collagen and autologous fat(2, 3). Acellular dermal matrix (ADM) is a kind of collagen matrix material with a three-dimensional spatial structure, which is obtained by removing immunogenic cell components of the epidermis and dermal tissue of humans or animals through a series of physicochemical and biological methods, and retaining the components and structures of the extracellular matrix such as collagen. ADM is widely used in the fields of burn, plastic surgery, surgery and wound repair(4-6). In our previous studies, we made cellular dermal matrix particles and explored acellular dermal matrix as a potential injectable filler material(7).

Stem cells are found in various tissues of the body and are responsible for internal environmental stability. Adipose-derived stem cells are favored by researchers because of their large amount of extraction, wide range of sources and low immunogenicity(8). In the study of yu et al., human adipose-derived stem cells (hADSCs) have been identified as a promising candidate for cell-assisted therapy to improve graft survival(9). So, we looked forward to applying this function of hADSC to filling materials. In this study, we explored the role of hADSC in filling material. For this purpose, the rat subcutaneous soft tissue was filled with ADM combined with autologous fat particles in different concentrations, and

hADSC was added to these filling materials. Then, we observed the filling condition and compared the filling effects of these different filling materials.

Methods

Culturing and Identifying of Human Adipose-Derived Stem Cells (hADSC).

We harvested the human subcutaneous fat tissue of healthy donors who had liposuction. The use of human adipose tissue was approved by the Ethics Committee of Plastic Surgery Hospital. The fresh fat specimen was washed three times with PBS containing 1% penicillin/streptomycin. The fat tissue was digested with 0.25% collagenase type I (Sigma, USA) at 37℃ for 0.5h and centrifuged at 1000 rpm for 5 min. The cell pellet was resuspended and filtered through a 70-μm filter (Corning, USA). After another centrifugation for 5 min, the cell pellet was resuspended in the culture medium consisting of Human Mesenchymal Stem Cell Medium (MSCM, ScienCell, USA) high glucose. The culture medium was replaced 48h after seeding to remove nonadherent cells; thereafter, the medium was replenished every 2–3 days. The ASCs were passaged 3 times for experiments. The hADSCs immunophenotype was analyzed by flow cytometry using the flow cytometer FACSCalibur (BD, New Jersey, USA). hADSCs cultivated to passage 3 were labeled with Human MSC Analysis Kit (BD, New Jersey, USA). Cell samples were labeled with each antibody separately and after processing, concentrated cell populations were gated and then the percentage of cells labeled with the selected antibodies was identified, as proposed by the International Federation for Adipose Therapeutics and Science (IFATS) and International Cell Therapy Society(10). The data use isotype as a reference and analysis were performed using the FlowJo7.6.1 Software.

Filler Preparation and Grouping

All animal study protocols and procedures were approved by the Animal Care Ethics Committee of Plastic Surgery Hospital. The animals used in this study included 12 adult male Sprague–Dawley rats weighing 350-450g. Rats were placed under general anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The rat was supine with the groin exposed. Open the skin along the groin to reveal a large amount of milky adipose tissue. The adipose tissue was carefully removed and the hemostasis was complete. The surgical wound was closed with 4-0 nylon sutures, and the rat was changed to the prone position. Fillers were then injected into the rat's back (Figure 1A). The whole process is carried out under aseptic conditions.

The adipose tissue was loaded into a 20ml spiral syringe, and cut into pieces with scissors. A small amount of PBS was added during the cutting process for lubrication. Then adipose tissue passe through the 1.0mm aperture converter and loaded into the 15ml centrifuge tube. After centrifugation at 1 000 r/min for 3 min, the upper layer was lipid droplets, the lower layer was PBS, blood and fibrous tissue, and the middle layer was intact fat particles (Figure 1B). The fat particles were loaded into a 15ml centrifuge tube for later use (Figure 1C).

Porcine acellular dermal matrix (PADM) particles (#6846, Unitrump Bio, Qidong, China), autologous fat particles, hADSC and PBS were combined with six different combinations were filled in the rat's back. They were mixed in the proportions given in Table 1. 18-gauge needles were used to inject each rat subcutaneously at 6 spots, each of the filling material contained a volume of 1.2 ml.

Table 1

Filling ratio

	A	B	C	D	E	F
Acellular dermal matrix	0ml	0ml	0.4ml	0.4ml	0.8ml	0.8ml
Autologous fat particles	0.8ml	0.8ml	0.4ml	0.4ml	0ml	0ml
hADSC	0ml	0.4ml	0ml	0.4ml	0ml	0.4ml
PBS	0.4ml	0ml	0.4ml	0ml	0.4ml	0ml

Assessment of the Filling Material Survival Rates

After 2, 6 and 12 weeks, four rats were selected randomly and euthanized with approved IACUC protocol, and the filling materials were carefully dissected from their back. Each filling material was measured, and the survival ratio for filling material was calculated by using the formula: survival volume/previous volume. Subsequently, each filling material was placed in paraformaldehyde and used for histological and immunohistochemistry examination.

Histological Analysis

The filling material transplants from the 6 groups were embedded in paraffin and cryopreserved in optimal cutting temperature (OCT) media compound. Paraffin-embedded filling material histological sections (5 μm) were prepared and then stained with H&E (PH1732, Phygene, Fuzhou, China). The H&E staining procedure was performed according to standard instructions. The microscope was used to obtain images. Neovascularization was assessed by counting the capillaries in 10 fields of each slide. The images were analyzed by Image Pro Plus software.

Statistical Analysis

All data were presented as the means ± standard error of the mean (SEM). GraphPad Prism 6.0 (USA) was used for statistical analysis and draft graphs. The Student's t test was used to evaluate the significant differences between the 2 groups. Multigroup comparisons were determined by one-way ANOVA with Bonferroni correction. Multigroup comparisons with different times were analyzed by two-way ANOVA with factors of treatment and time. Intergroup differences were analyzed by performing post

hoc Bonferroni tests. P values ≤ 0.05 were considered statistically significant (### or ***, P ≤ 0.001; **, P ≤ 0.01; *, P ≤ 0.05).

Results

Culturing and Identifying of Human Adipose-Derived Stem Cells (hADSC).

hADSCs were successfully isolated from the fat tissue. Cells adhered to the plastic culture flask. In passage 0, hADSCs grew adhering to the wall and presented a fibroblast-like cell morphology, and the circular suspension cells were other cells or inactivated adipose stem cells. During sub-culturing up to passage three, the morphologic or growth pattern didn't change (Fig. 2A). The stem cell markers CD73, CD90, and CD105 were highly expressed (> 90%) in our cells, and the cells had negative expression (≤ 3%) of the negative cocktail markers (CD11b, CD19, CD34, CD45, HLA-DR) (Figure 2B). The result of flow cytometry showed cells were stem cells(10).

Filling Material Survival Rates

At 2, 6 and 12 weeks after subcutaneous injection of the filling material, four rats were selected randomly and killed. The subcutaneous fillers were under the skin of the rats' back, and they were extracted for assessment of the changes in volume. Macroscopic images of filling materials at three different time points are shown in Figure 3.

The volume of different filling materials were detected at 2, 6 and 12 weeks after subcutaneous injection to calculate the survival rate. All survival rate curves of different fillers were shown in figure 4. In the fat particles with PBS group and fat particles with hADSC group, the two groups were statistically different at six weeks (P□0.05). At 12 weeks, the fat particles with PBS or hADSC cannot be found, and the survival rate was 0. In the fat particles and PADM with PBS or hADSC group, the two groups were statistically different at 6 and 12 weeks (P□0.01). In the PADM with PBS or hADSC group, the two groups were statistically different at three time points (P□0.05). In short, the survival rate of filler with hADSC group was higher than the PBS group after 6 weeks. In the three PBS group, survival rates of the PADM group were higher than the other two groups at three time points, and survival rates of the fat particles group were lowest. In the three hADSC groups, the results were similar to the three PBS groups.

Histological Analysis

The H&E staining results performed to examine angiogenesis and cell infiltration in each group. As shown in figure 5, the H&E staining of the filler materials at 2 and 6 weeks showed that there were limited inflammatory cell infiltration and neovascularization in all groups (microbars 20 μm). The staining of the filler materials at 12 weeks revealed that there was almost no inflammatory cell infiltration, and the neovascularization significantly increased compared to 2 and 6 weeks.

The results of angiogenesis analysis were shown in the figure 6. In the fat particles with PBS group and fat particles with hADSC group, the number of blood vessels in the PBS group was 5.50 ± 0.29 at 2

weeks, and 7.25 ± 0.63 at 6 weeks, the number of blood vessels in the hADSC group was 8.75 ± 1.49 at 2 weeks, and 9.75 ± 0.48 at 6 weeks. The two groups were statistically different at 2 weeks (P□0.05), but the two groups were not statistically different at 6 weeks. In the fat particles and PADM with PBS or hADSC group, the number of blood vessels in PBS group was 6.00 ± 0.41 at 2 weeks, 8.25 ± 0.48 at 6 weeks, 9.00 ± 0.41 at 12 weeks, and the number of blood vessels in the hADSC group was 8.50 ± 0.65 at 2 weeks, 10.25 ± 0.48 at 6 weeks, 12.50 ± 0.65 at 12 weeks. The two groups were statistically different at 2, 6 and 12 weeks (P□0.05). In the PADM with PBS or hADSC group, the number of blood vessels in PBS group was 1.25 ± 0.250 at 2 weeks, 5.50 ± 0.65 at 6 weeks, 8.25 ± 0.48 at 12 weeks, and the number of blood vessels in hADSC group was 4.00 ± 0.41 at 2 weeks, 8.75 ± 1.25 at 6 weeks, 11.50 ± 0.65 at 12 weeks. The two groups were statistically different at 2, 6 and 12 weeks (P□0.05). In the three PBS groups, the number of blood vessels in the PADM group was lower than the other two groups at 2 and 6 weeks. There was no significant difference between the combined group and PADM groups at 12 weeks. In the three hADSC groups, the number of blood vessels in the PADM group was lower than the other two groups at 2 weeks, and no significant difference between the combined group and PADM groups at 12 weeks.

Discussion

As a new therapeutic method, cell therapy has been applied in many fields(11, 12). Mesenchymal stem cells are the most popular choice for cell therapy. MSCs may regulate immune response by releasing soluble immunosuppressive factors(13), while adipose-derived stem cells can be used as cell therapy in various animal experimental models due to their wide sources and large extraction volume. In the study of Lee et al., hADSCs were used to repair rat tendons, and hADSC could promote tendon healing and survive for at least four weeks(11). There is a study has shown that hADSCs can locally survive for 3 months or even 6 months in a rat model(14). Therefore, we set the longest observation time point as 12 weeks in our study. No serious complications occurred in rats during our experiment, which reflects the feasibility of hADSCs xenotransplantation.

Fat transplantation is a safe and effective injection filling method. Since autologous adipose tissue is easily obtained through liposuction, fat transplantation has become the preferred treatment for clinical soft tissue enhancement and reconstruction, and has been applied in various cosmetic or reconstruction indications(15, 16). However, fat transplantation has the disadvantage of a high absorption rate leading to a poor long-term prognosis. The survival rate of fat transplantation is between 20 and 80% (17). The loss of fat volume and function is caused by apoptosis and necrosis of fat cells and inadequate and premature revascularization(18). Recently, assisted fat transfer with human adipose-derived stem cells has been shown to significantly improve the survival rate of fat transplantation(19, 20). Our experimental results are also consistent with this conclusion. In terms of survival rate, we found that hADSCs significantly improved the survival rate in each group at 6 and 12 weeks from our results. However, the fat particles groups were fully absorbed at 12 weeks, and the long-term effects of hADSCs in this group could not be determined. In terms of vascular density, hADSCs significantly increased the vascular density in the fat particles-PADM mixture group and PADM group at three time points, but hADSCs did not have a significant effect in the fat particles group.

Acellular dermal matrix is characterized by rapid vascularization, low antigenicity and stable performance. In our previous studies, the acellular dermal matrix was prepared in granular form for injection filling and we found that it was characterized by high stability, low inflammatory response, and low absorption rate(7). In this study, acellular dermal matrix particles prepared were selected for further study. With or without hADSCs, the PADM group had a higher survival rate than the other two groups. The survival rate of the PADM group with hADSCs was significantly higher than the combined group at 2 and 6 weeks. However, there was no significant difference between the PADM group and the combined group in the survival rate. We speculated that the local number of hADSCs decreased in the filling materials at 12 weeks, which may be related to their migration, which is consistent with the results of the study by Haddad-Mashadrizeh A et al(14). In terms of vascular density, the fat particles group and the combined group had higher vascular density than the PADM group at 2 and 6 weeks. There was no statistical difference between the filling materials with the assistance of hADSCs at 6 and 12 weeks. In short, hADSCs had the greatest effect on vascular density in the PADM group at 6 weeks.

Conclusion

hADSCs improved the survival rate of the fat particles group, the combined group and the PADM group at 6 and 12 weeks in rats. hADSCs increased the vascular density in the combined group and PADM group at three time points in rats.

Declarations

Acknowledgements:

Thanks to all members of the 2nd Department of Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College.

Funding:

This study was supported by the Discipline Construction Project of Peking Union Medical College (201920200401) and CAMS Innovation Fund for Medical Sciences (2021-I2M-1-068).

Compliance with Ethical Standards.

Conflict of interest:

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

All experiments conducted in this study were reviewed and approved by the Local Animal Ethics Committee (No. 202003003). The use of human adipose tissue was approved by the Ethics Committee of Plastic Surgery Hospital (No. 2150019022). All participants provided informed consent.

Availability of data and materials:

All data generated or analysed during this study are included in this published article and its supplementary information files.

References

1. Winslow C. Filling the Midface: Injectables. Facial Plast Surg. 2016;32(5):473-9.
2. de Maio M, Swift A, Signorini M, Fagien S, Aesthetic Leaders in Facial Aesthetics Consensus C. Facial Assessment and Injection Guide for Botulinum Toxin and Injectable Hyaluronic Acid Fillers: Focus on the Upper Face. Plast Reconstr Surg. 2017;140(2):265e-76e.
3. Zheng ZF, Hao YS, Yin JFY, Lei XX, Cheng BA, Huang WH. Autogenous Fat Transplantation and Botulinum Toxin Injection Into the Masseter Muscle to Create an Ideal Oval Face. Aesthet Surg J. 2021;41(6):Np579-Np88.
4. Margulies IG, Salzberg CA. The use of acellular dermal matrix in breast reconstruction: evolution of techniques over 2 decades. Gland Surg. 2019;8(1):3-10.
5. Wainwright DJ, Bury SB. Acellular Dermal Matrix in the Management of the Burn Patient. Aesthet Surg J. 2011;31(7):13s-23s.
6. Reyzelman AM, Bazarov I. Human acellular dermal wound matrix for treatment of DFU: literature review and analysis. J Wound Care. 2015;24(3):128; 9-34.
7. Qu S, Yi J, Chen Z, Zhou J. A Potential Filling Material for Wound Healing and Shaping: Acellular Dermal Matrix Combined with Autologous Dermis. Aesthetic Plast Surg. 2021;45(2):740-8.
8. Mazini L, Rochette L, Admou B, Amal S, Malka G. Hopes and Limits of Adipose-Derived Stem Cells (ADSCs) and Mesenchymal Stem Cells (MSCs) in Wound Healing. Int J Mol Sci. 2020;21(4).
9. Yu F, Witman N, Yan D, Zhang S, Zhou M, Yan Y, et al. Human adipose-derived stem cells enriched with VEGF-modified mRNA promote angiogenesis and long-term graft survival in a fat graft transplantation model. Stem Cell Res Ther. 2020;11(1):490.
10. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7.
11. Lee SY, Kwon B, Lee K, Son YH, Chung SG. Therapeutic Mechanisms of Human Adipose-Derived Mesenchymal Stem Cells in a Rat Tendon Injury Model. Am J Sports Med. 2017;45(6):1429-39.
12. Chen S, He Z, Xu J. Application of adipose-derived stem cells in photoaging: basic science and literature review. Stem Cell Res Ther. 2020;11(1):491.
13. Wang J, Liao L, Tan J. Mesenchymal-stem-cell-based experimental and clinical trials: current status and open questions. Expert Opin Biol Ther. 2011;11(7):893-909.
14. Haddad-Mashadrizeh A, Bahrami AR, Matin MM, Edalatmanesh MA, Zomorodipour A, Gardaneh M, et al. Human adipose-derived mesenchymal stem cells can survive and integrate into the adult rat eye

following xenotransplantation. Xenotransplantation. 2013;20(3):165-76.
15. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Autologous fat transplantation for breast reconstruction: A literature review. Ann Med Surg (Lond). 2016;12:94-100.
16. Borrelli MR, Patel RA, Sokol J, Nguyen D, Momeni A, Longaker MT, et al. Fat Chance: The Rejuvenation of Irradiated Skin. Plast Reconstr Surg Glob Open. 2019;7(2):e2092.
17. Zhu M, Cohen SR, Hicok KC, Shanahan RK, Strem BM, Yu JC, et al. Comparison of three different fat graft preparation methods: gravity separation, centrifugation, and simultaneous washing with filtration in a closed system. Plast Reconstr Surg. 2013;131(4):873-80.
18. Eto H, Kato H, Suga H, Aoi N, Doi K, Kuno S, et al. The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. Plast Reconstr Surg. 2012;129(5):1081-92.
19. Kolle SF, Fischer-Nielsen A, Mathiasen AB, Elberg JJ, Oliveri RS, Glovinski PV, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. Lancet. 2013;382(9898):1113-20.
20. Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. Regenerative Medicine. 2009;4(2):265-73.

Figures

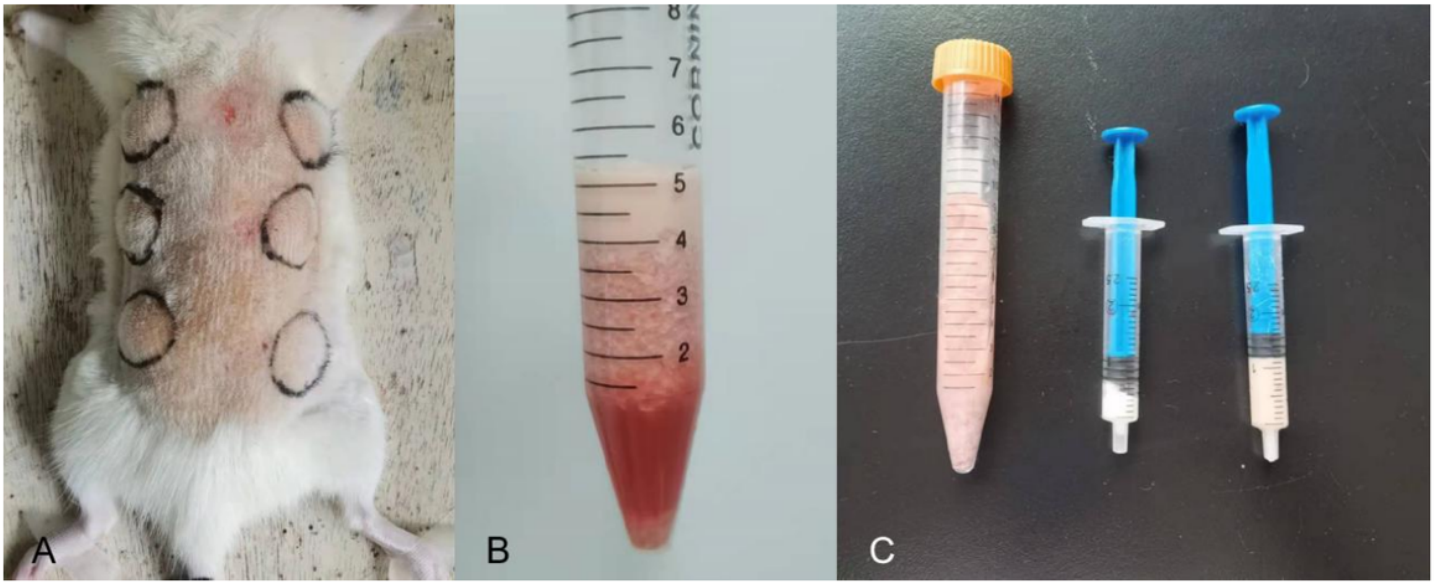

Figure 1

Gross observation of filling and preparation of filling material. (A) The gross observation of six different filling material filled into rat's back. (B) The adipose tissue was cut and centrifuged, the upper layer was lipid droplets, the lower layer was PBS, blood and fibrous

tissue, and the middle layer was intact fat particles. (C) The left is autologous fat particles, the middle is acellular dermal matrix, and the right is equal mixture of autologous fat particles and acellular dermal matrix.

Figure 2

Morphology and immunophenotype characteristics of hADSCs. (A) Representative microscopic illustrations of hADSCs. Scale bar 250 μm. (B) Representative histograms from the flow cytometry analysis showing surface marker expression on hADSCs, monoclonal antibody control (red) and the stained cells (blue).

Figure 3

Macroscopic images of subcutaneous filler materials.

( ) Macroscopic images of the harvested filler materials 2 weeks post transplantation. ( ) Macroscopic images of the harvested filler materials 2 weeks post transplantation. ( ) Macroscopic images of the harvested filler materials 6 weeks post transplantation. ( ) Macroscopic images of the harvested filler materials 12 weeks post transplantation.

Figure 4

The survival rate curve of different filler.

All data are expressed as the mean ± SD (number from each group = 4; two-way ANOVA analysis followed post hoc multiple comparisons with a Bonferroni correction).

(A) The survival rate curve of fat particles with PBS or hADSC. *P < 0.05, vs. Fat particles and PBS group. (B) The survival rate curve of fat particles and acellular dermal matrix with PBS or

hADSC. **P < 0.01, ****P < 0.0001, vs. Fat particles, acellular dermal matrix and PBS group. (C) The survival rate curve of acellular dermal matrix with PBS or hADSC. *P < 0.05, **P < 0.01, vs. Acellular dermal matrix and PBS group. (D) The survival rate curve of fat particles, acellular dermal matrix and combined filler with PBS. ****P< 0.0001, ##P < 0.01, ####< 0.0001, vs. Fat particles and PBS group. (E) The survival rate curve of fat particles, acellular dermal matrix and combined filler with hADSC. ****P< 0.0001, ####< 0.0001, vs. Fat particles and hADSC group.

Figure 5

H&E staining results Scale bar: 25 μm.(A) H&E staining results of the filler 2 weeks after injection. (B) H&E staining

results of the filler 6 weeks after injection. (C) H&E staining results of the filler

12 weeks after injection.

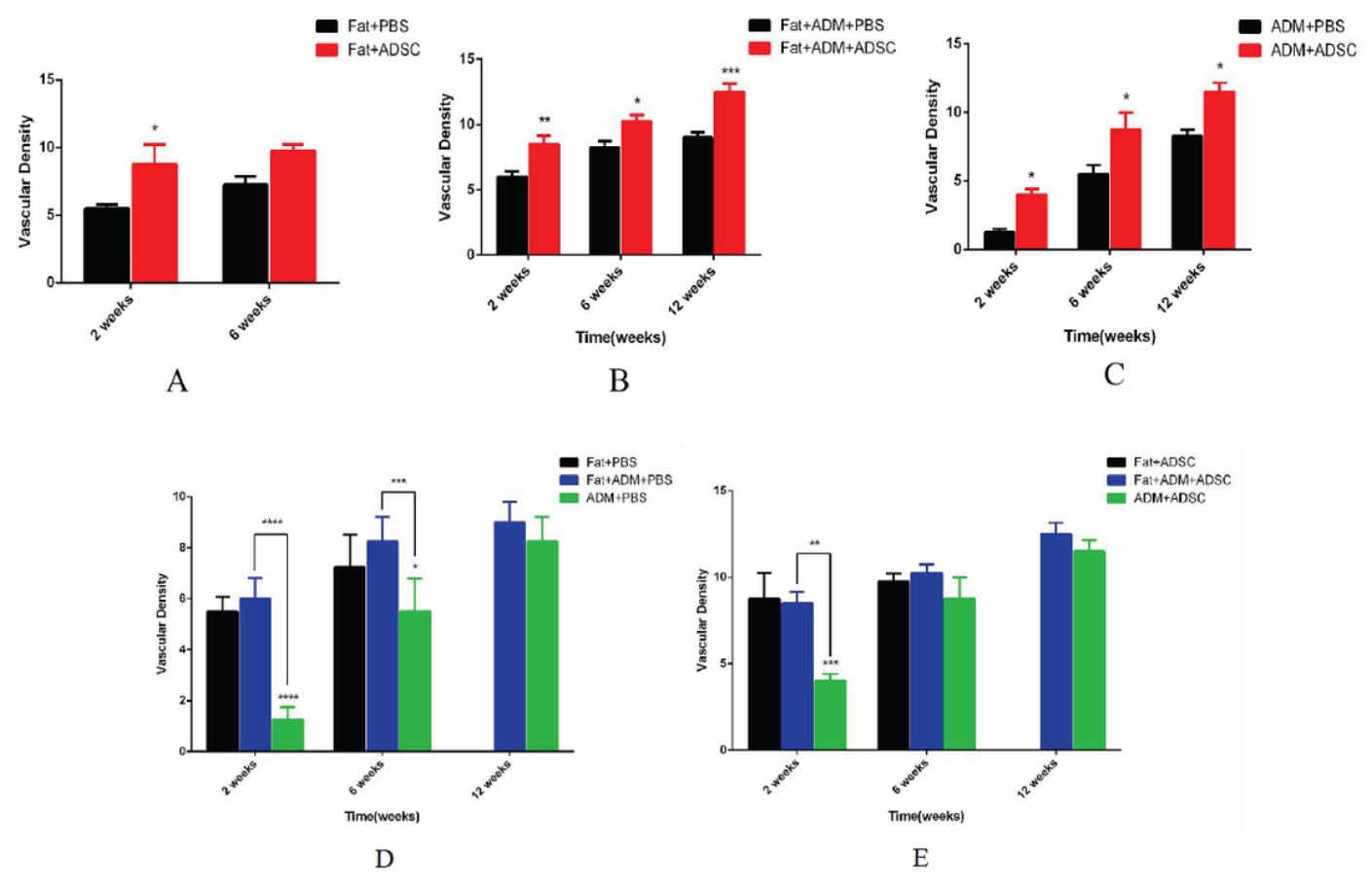

Figure 6

Assessment of the density of blood vessels at 2, 6 and 12 weeks after filler injection.

(A): Histogram showing the density of blood vessels in fat particles with PBS or hADSC group. *P < 0.05, vs. Fat particles and PBS group. (B) Histogram showing the density of blood vessels in fat particles and acellular dermal matrix with PBS or hADSC group. *P < 0.05, **P < 0.01, ***P < 0.001, vs. Fat particles, acellular dermal matrix and PBS group. (C) Histogram showing the density of blood vessels in acellular dermal matrix with PBS or hADSC group. *P < 0.05 vs. Acellular dermal matrix and PBS group. (D) Histogram showing the density of blood vessels in fat particles, acellular dermal matrix and combined filler with PBS group. *P < 0.05, ***P < 0.001, ****P< 0.0001 vs. Fat particles and PBS group. (E) Histogram showing the density of blood vessels in fat particles, acellular dermal matrix and combined filler with hADSC group. **P < 0.01, ***P < 0.001, ****P< 0.0001 vs. Fat particles and hADSC group.