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Human amniotic mesenchymal stem cells improve patency and regeneration of electrospun biodegradable vascular grafts via anti-thrombogenicity and M2 macrophage polarization

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ABSTRACT

Small-diameter vascular grafts (SDVGs) are prone to thrombosis and have low long-term patency rates for various reasons, which cannot meet the clinical requirements. In this work, Human amniotic mesenchymal stem cell (hAMSC) seeding electrospun polylactic acid-co-polycaprolactone (PLCL) SDVGs are fabricated and their application potential is systematically evaluated. The SDVG has excellent mechanical properties. PLCL eletrospinning membrane has no cytotoxicity. The SDVG has a porous fibrous tube wall, uniform distribution of hAMSCs, and good cell compatibility, blood compatibility, histocompatibility and mechanical properties. hAMSCs loading can improve the acute antithrombotic ability, patency and in vivo regeneration effect of PLCL electrospun SDVGs. The mechanism is related to hAMSCs increasing the content of endothelial cells, contractile smooth muscle cells, and M2 macrophages, as well as activating extracellular matrix production.

1. Introduction

According to World Health Organization (WHO) reports, there are estimated 17.9 million patients died from cardiovascular disease all over the world, and the death caused by cardiovascular disease accounts for more than 40 % of the total deaths of urban and rural residents, so the prevention and treatment are imminent [1]. Among them, the treatment of severe obstructive cardiovascular diseases (such as coronary heart disease and severe lower limb ischemia) require vascular transplantation [2,3]. At present, the gold standard of vascular transplantation is autologous blood vessels, such as great saphenous vein, internal mammary artery and radial artery, which have good compatibility and high long-term patency rate [4]. However, obtaining autologous blood vessels causes great damage to patients, and also about one-third of patients are difficult to obtain due to physical conditions and vascular injuries [5,6]. The existing large caliber vascular grafts in

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the market have been applied in clinic and achieved satisfactory results [7], but the SDVGs are prone to thrombosis after transplantation due to their small caliber, slow blood flow and low blood pressure, leading to a low long-term patency rate, which cannot meet the clinical needs [8]. Therefore, there is a huge clinical gap in the SDVG field.

To solve this problem, researchers have conducted a lot of research on restraining thrombosis, and there have been two the main strategies [9]: material anticoagulant compound modification and cell lining. The antithrombotic properties of the modified materials decrease with time, while the cell lining is expected to maintain or even enhance the antithrombotic properties due to the self-renewal and paracrine functions of cells. The purpose of the cell lining strategy is to ultimately form a stable and complete endothelial cell layer in the vascular intima. The normal endothelial cell layer has important physiological roles, regulating physiological processes such as thrombosis and smooth muscle cell proliferation by secreting a variety of cytokines [10–12].

A variety of cells have been explored for the antithrombotic modification of SDVGs. Endothelial cells become the first choice because of their natural antithrombotic function, but due to the limited proliferation ability of mature endothelial cells and the difficulty of autologous acquisition, they are not the best one [13]. In recent years, researchers have found that a variety of stem cells can be used as an excellent choice for antithrombotic modification of SDVG [14]. Due to ethical disputes and tumorigenicity of embryonic stem cells [15], and gene safety and transformation efficiency of induced pluripotent stem cells [16], adult stem cells have become the main research direction [17]. Among them, bone marrow mesenchymal stem cells and adipose derived mesenchymal stem cells are the most promising ones, but their acquisition still needs invasive operation, and the quality of cells greatly vary with individual health status [17].

Recently, researchers turned their attention to a new type of placenta derived stem cells–human amniotic mesenchymal stem cells (hAMSCs) [18,19]. hAMSC has the basic properties of adult stem cells, such as multi-directional differentiation, self-renewal, no tumorigenicity, low immunogenicity and so on [20]. Their source is special, and the acquisition process is non-invasive, without ethical and moral restrictions [21]. They can be efficiently obtained with high quantity and high purity [22]. Compared to adult dereived stem cells, placenta-derived hAMSCs have stronger abilities on expansion, differentiation and immune regulation. hAMSCs are able to differentiate into vascular endothelial cells and smooth muscle cells, and enhance the proliferation and adhesion ability of vascular endothelial cells in vitro [23–26].

The scaffold material of SDVGs also has an important impact on the patency rate. Recent studies have shown that electrospinning of degradable polymers is the key direction of the next generation of vascular graft materials [27]. Poly(lactic acid-co-polycaprolactone) (PLCL) electrospinning scaffolds have non-toxicity, biocompatibility, and appropriate degradation speed, with adjustable mechanical properties and extracellular matrix structure similarity [28,29]. PLCL electrospinning scaffolds are also simple to prepare, easy to scale, has large specific surface area and porosity, and conducive to the regeneration of vascular tissue [30,31], which have achieved good results in preclinical trials [32–34].

The present work aims to implant hAMSCs into PLCL electrospun tubular scaffolds to construct a new SDVG (hAMSC PLCL vascular graft), explore its in vitro and in vivo performance and the role of hAMSCs in vascular graft remodeling, which would provide an experimental and theoretical basis for subsequent preclinical evaluation and clinical application.

2. Materials and methods

For detailed information, please see the Supplemental Material S1.

3. Results

3.1. Morphological characteristics and phenotype identification of hAMSCs

The hAMSCs cultured in the 3rd generation presented as long spindle-shaped fibroblast-like morphologies, with local vortice-like growth arrangement, which is a significant feature of MSCs (Fig. 1A). Immunostaining identification of mesenchymal phenotype showed that almost all cells had strong vimentin-positive brown staining in the cytoplasm (Fig. 1B). The expression levels of immune phenotype cell surface markers CD44, CD73, CD90, and CD105 exceeded 95 %, while the expression levels of non-MSC surface markers such as CD45, CD19, CD34, CD11b, and HLA-DR were less than 2 %, which meets the definition and standards of MSCs by the International Society of Mesenchymal Stem Cells (Fig. 1C). The hAMSCs had the potential for multidirectional differentiation and could differentiate into osteoblasts and adipocytes under appropriate induction. Osteogenic induction of hAMSCs resulted in a large amount of orange red calcium salt deposition (Fig. 1D), while adipogenic induction of hAMSCs resulted in a large amount of red lipid droplets (Fig. 1E).

3.2. Vascular graft structural characterization

PLCL vascular grafts were successfully prepared by optimizing electrospinning conditions. Subsequently, the prepared PLCL vascular grafts were characterized in detail. By observing the surface of PLCL vascular grafts through SEM, it can be seen that the material fibers presented a random distribution with porous structures. The wall thickness of the vascular graft is 0.51 ± 0.05 mm and the inner diameter is 2.19 ± 0.05 mm, as measured by a digital micrometer thickness gauge and a vernier caliper (Fig. 2A and B and Table 1). The fiber diameter and pore size of vascular grafts were statistically analyzed using ImageJ software, and the porosity was measured using liquid immersion method. The research results show that the fiber diameter of PLCL vascular grafts is 0.36 ± 0.10 μm, the pore size is 3.63 ± 1.66 μm, and the porosity is 69.74 % ± 5.81 % (Fig. 2C and Table 1). This porosity level is sufficient to promote nutrient exchange for cell growth.

3.3. hAMSC loading identification

hAMSCs were then loaded into PLCL vascular grafts. One day later, DAPI staining was performed, and the cross-sectional fluorescence results showed that the blue fluorescence of hAMSC cell nuclei densely surrounded the lumen and adhered evenly. The unfolding surface of the inner wall of the blood vessel also revealed a uniform distribution of cell nuclei (Figs. 2D and 2E), indicating the successful preparation of vascular grafts loaded with hAMSCs.

3.4. Mechanical performance analysis

From the stress-strain curve, the maximum longitudinal stress was calculated to be 7.83 ± 0.32 MPa, the longitudinal elastic modulus was 861.8 ± 293.2 kPa, the maximum longitudinal elongation at break was 778.37 % ± 137.12 %, the maximum circumferential stress was 2.11 ± 0.24 MPa, the circumferential elastic modulus was 2120 ± 200 kPa, and the maximum circumferential elongation at break was 270.90 % ± 40.91 % (Fig. 2F and Table 2). Its suture strength was 2.93 ± 0.17 N, and the burst pressure was 0.22 ± 0.01 MPa, which is greater than the human vein burst pressure of 1600 mmHg (0.213 MPa) (Table 2) [35]. The estimated compliance of the graft calculated from circumferential elastic modulus was 35.38 ± 3.33 %/100 mmHg, which is comparable to the polycaprolactone graft in our previous report [36], higher than the reported trilayered electrospun polyurethane-PLCL-gallic acid grafts [37] and gelatin-methacryloyl grafts with PCL/PLCL fiber reinforcement [38], and much higher than those of the PCL/PLCL grafts with random

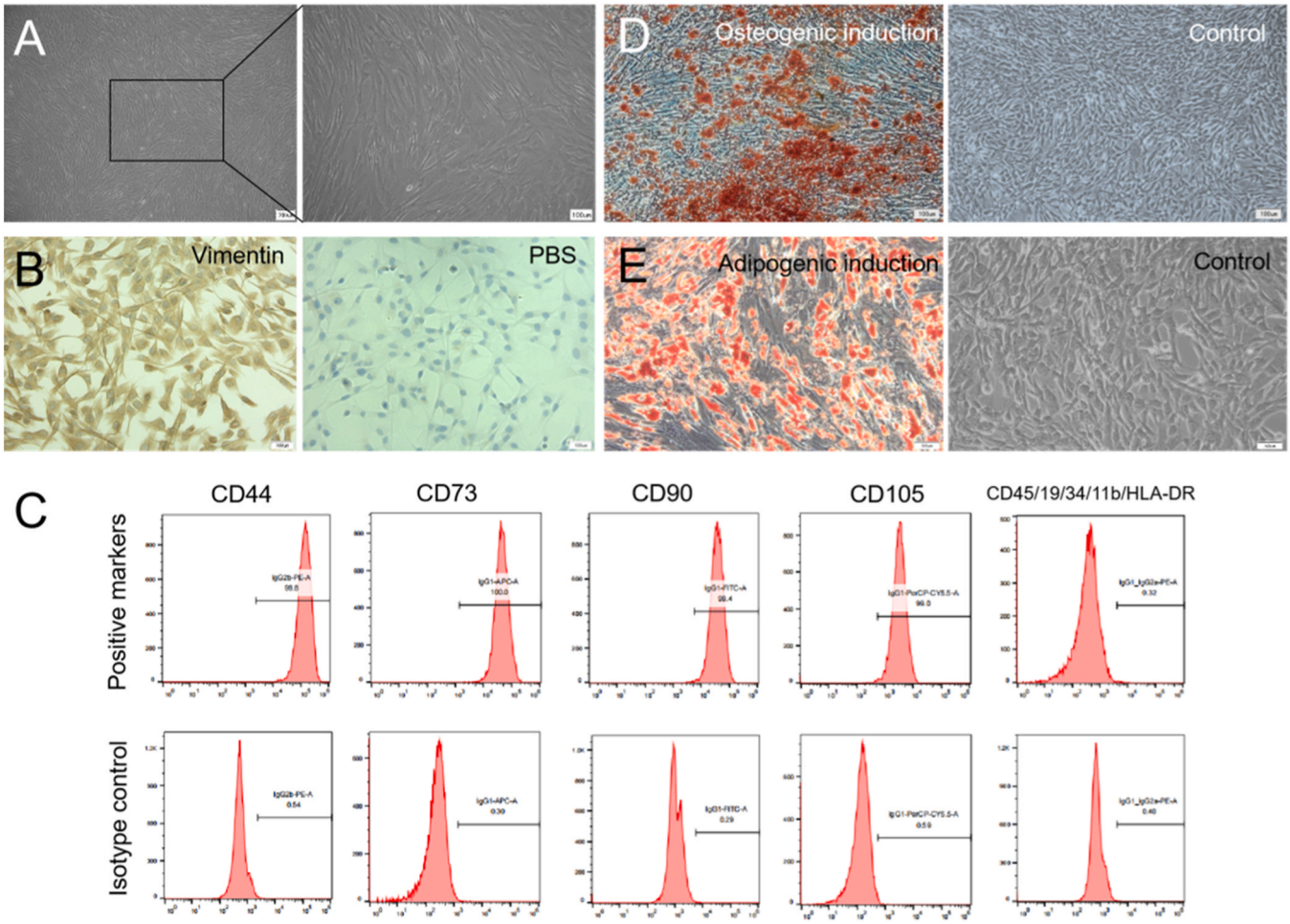

Fig. 1. Identification of hAMSCs. A: Morphology of hAMSCs, left: scale bar= 200 μm, right: scale bar= 100 μm, B: Vimentin expression, scale bar= 100 μm, C: Surface marker test of hAMSCs by flow cytometery, D: Osteogenic differentiation of hAMSCs, scale bar= 100 μm, E: adipogenic differentiation of hAMSCs, scale bar= 50 μm.

or oriented fibers [39], and Dacron and ePTFE grafts [40]. Although the estimated compliance suggests a very elastic and flexible vascular graft, the calculation could be further experimentally identified for more precise values. Collectively, the above results indicate that the mechanical properties of PLCL electrospun vascular grafts can meet the requirements for transplantation.

3.5. Cell compatibility analysis

As shown in Fig. 3A, hAMSCs proliferated on PLCL electrospun membrane, and on the 1st and 2nd day of culture, the cell density was not statistically different from that cultured on tissue culture plate (TCP) surface. On day 3, 4, and 5, the growth of hAMSCs on TCP was faster than that on PLCL membrane. On day 4, cell proliferation on PLCL membrane was significantly accelerated. On days 6 and 7, the cell density on PLCL membrane exceeded that of TCP (Fig. 3A). To observe cell adhesion, growth and viability, Live/Dead staining was used (Fig. 3B). The results of the live/dead staining experiment showed that there were almost no dead cells in the field of view. On the 1st, 4th, and 7th days of culture, the number of hAMSCs on the PLCL membrane and TCP increased gradually. On the 1st day, the cell spreading degree of the PLCL spinning membrane group was lower than that of the TCP group, and there was no significant difference on the 4th and 7th days. Based on the results, PLCL has no significant effect on the growth density, morphology, and activity of hAMSCs, which is non-toxic, and has good cell compatibility.

3.6. Blood compatibility analysis

There was no statistically significant difference in thrombin activity among groups cultured on the TCP substrate surface, PLCL spun film, TCP surface loaded with hAMSC, and PLCL spun surface loaded with hAMSC, indicating that hAMSC PLCL vascular grafts do not increase thrombin activity (Fig. 3C). Immunofluorescence staining showed that compared to the TCP group, there was no significant change in platelet adhesion in the other groups (Fig. 3D). The hemolysis experiment showed that there was no apparent difference in the color and transparency of the supernatant between the PLCL vascular graft group and the negative control group, while the transparency of the positive control group was poor (Fig. 3E). After calculation, the hemolysis rate of the vascular graft group is much lower than the safety threshold of 5 %, indicating that hAMSC PLCL vascular grafts will not increase hemolysis (Fig. 3F).

3.7. Histological compatibility evaluation

Two weeks after subcutaneous implantation, samples were explanted and HE staining results showed that hAMSC implantation led to more cell infiltration and extracellular matrix deposition of PLCL electrospun vascular graft (Fig. 4A). The scaffolds with and without hAMSCs both integrated well with the tissue without obvious inflammation or immune rejection, indicating good tissue compatibility. Masson staining showed that the hAMSC loading group had more muscle fibers on the

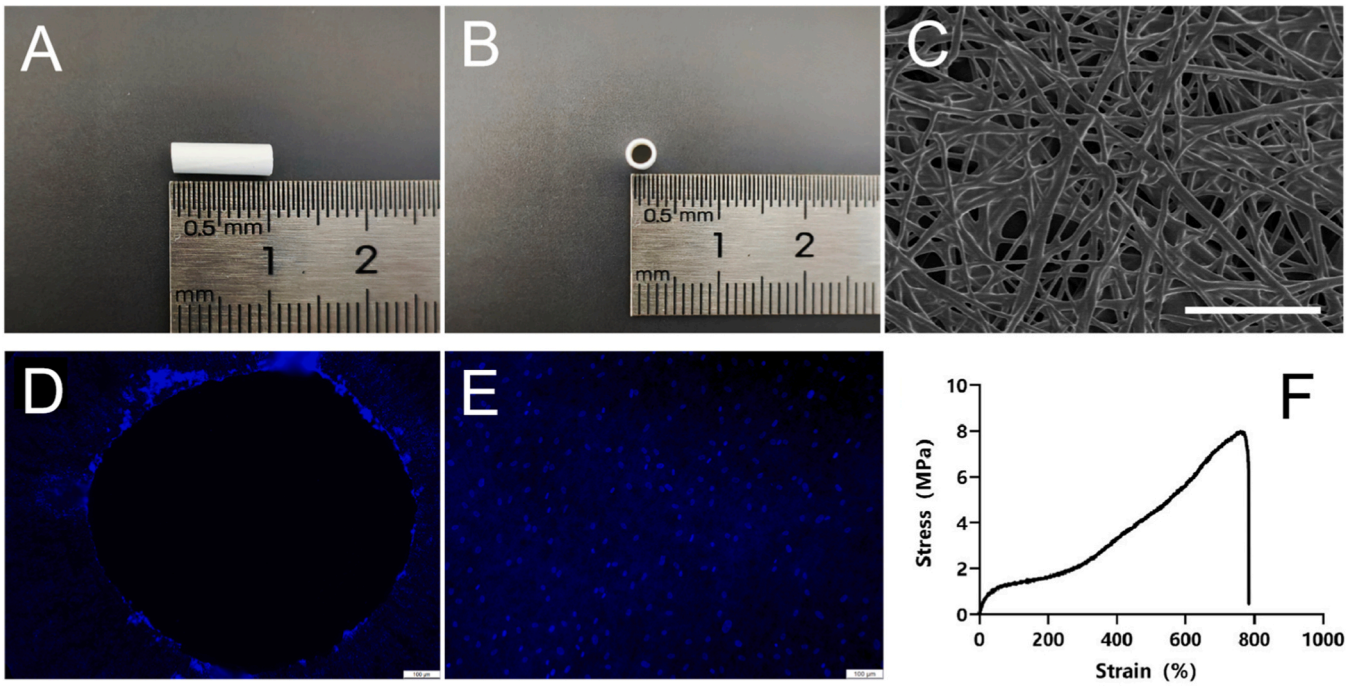

Fig. 2. Morphology and mechanical property of the vascular graft. A: Side view of the VG, B: Cross-section view of the VG, C: SEM of the VG wall, scale bar= 20 μm, D: DAPI staining of the cross section of the VG, scale bar= 100 μm, E: DAPI staining of the inside surface of the VG, scale bar= 100 μm, F: The representative longitudinal stress-strain curve of the VG.

Table 1
Key Morphological Characterization of PLCL Vascular Graft.

Morphological parameter	Value (mean ± SD)
Fiber diameter	0.36 ± 0.10 μm (n = 100)
Pore size	3.63 ± 1.66 μm (n = 50)
Wall thickness	0.51 ± 0.05 mm (n = 3)
Inner diameter	2.19 ± 0.05 mm (n = 3)
Porosity	69.74 % ± 5.81 % (n = 3)

Table 2
Key Mechanical Properties of hAMSC PLCL Vascular Graft.

Mechanical parameter	Value (n = 3, mean ± SD)
Longitudinal elastic modulus	861.8 ± 293.2 kPa
Longitudinal burst stress	7.83 ± 0.32 MPa
Longitudinal elongation at break	778.37 % ± 137.12 %
Circumferential elastic modulus	2120 ± 200 kPa
Circumferential burst stress	2.11 ± 0.24 MPa
Circumferential elongation at break	270.90 % ± 40.91 %
Suture retention	2.93 ± 0.17 N
Burst pressure	0.22 ± 0.01 MPa

outer surface of the vascular grafts and collagen inside, while the group without hAMSC implantation had fewer (Fig. 4A). EVG staining showed that the vascular grafts without hAMSC implantation had less elasticity and collagen fibers, but increased after implantation (Fig. 4A). hAMSCs increased the deposition of elastic fibers. After 14 days, both groups of vascular grafts showed no calcification (Fig. 4A). Safranin O staining showed that vascular grafts implanted with hAMSC had more deposition of mucopolysaccharides (Fig. 4A).

3.8. Acute thrombosis test

Blood clots adsorption was estimated by weighing the vascular grafts before and after 2 hours of in vivo circulation. Photo analysis revealed that the PLCL vascular grafts without hAMSC implantation had thrombus formation, while those with hAMSC implantation had fewer thrombi (Fig. 4B). The average mass increase of cell-free PLCL vascular graft was 13.43 mg, while that of the cell-containing was 7.53 mg. Loading hAMSCs reduced the mass by 5.9 mg, and also the *t*-test P value between groups was 0.077, quite close to 0.05 (Fig. 4C). SEM observation showed that there was a large amount of protein aggregation, a small amount of red blood cells, and activated platelets in the vascular graft lumen without hAMSC implantation, while there was only a very small amount of inactive platelets on the surface of the lumen with hAMSC implantation (Fig. 4D). The results indicate that the implantation of cell-free PLCL vascular grafts during the acute phase activates a small number of platelets, while hAMSC loading can inhibit platelet activation and thrombus formation.

3.9. Analysis of in vivo patency of vascular grafts

Two rats died in the cell-free PLCL vascular graft group at 8th and 14th days after implantation. After dissection, it was found that these two cases of vascular grafts had occlusion caused by thrombosis. The remaining three rats in the cell-free group still survived well after one month of circulation, and their vascular grafts were found to be unobstructed after sampling. In contrast, the rats of PLCL vascular graft group loaded with hAMSCs survived well after one month, and all vascular grafts were found to be unobstructed after sampling. The hAMSC PLCL vascular grafts were all unobstructed, achieving a patency rate of 100 %, while the cell-free groups only had a patency rate of 60 % (Fig. 5A). The data show that the introduction of hAMSCs effectively improves the patency rate of PLCL electrospun vascular grafts.

3.10. Analysis of in vivo remodeling of vascular grafts

HE staining showed that compared to cell-free grafts, the cell infiltration and the expression of extracellular matrix of hAMSC loading PLCL vascular grafts were deeper and denser, forming a richer inner membrane layer that was closer to natural blood vessels (Fig. 5B). Both groups had no obvious inflammatory cell infiltration, indicating good

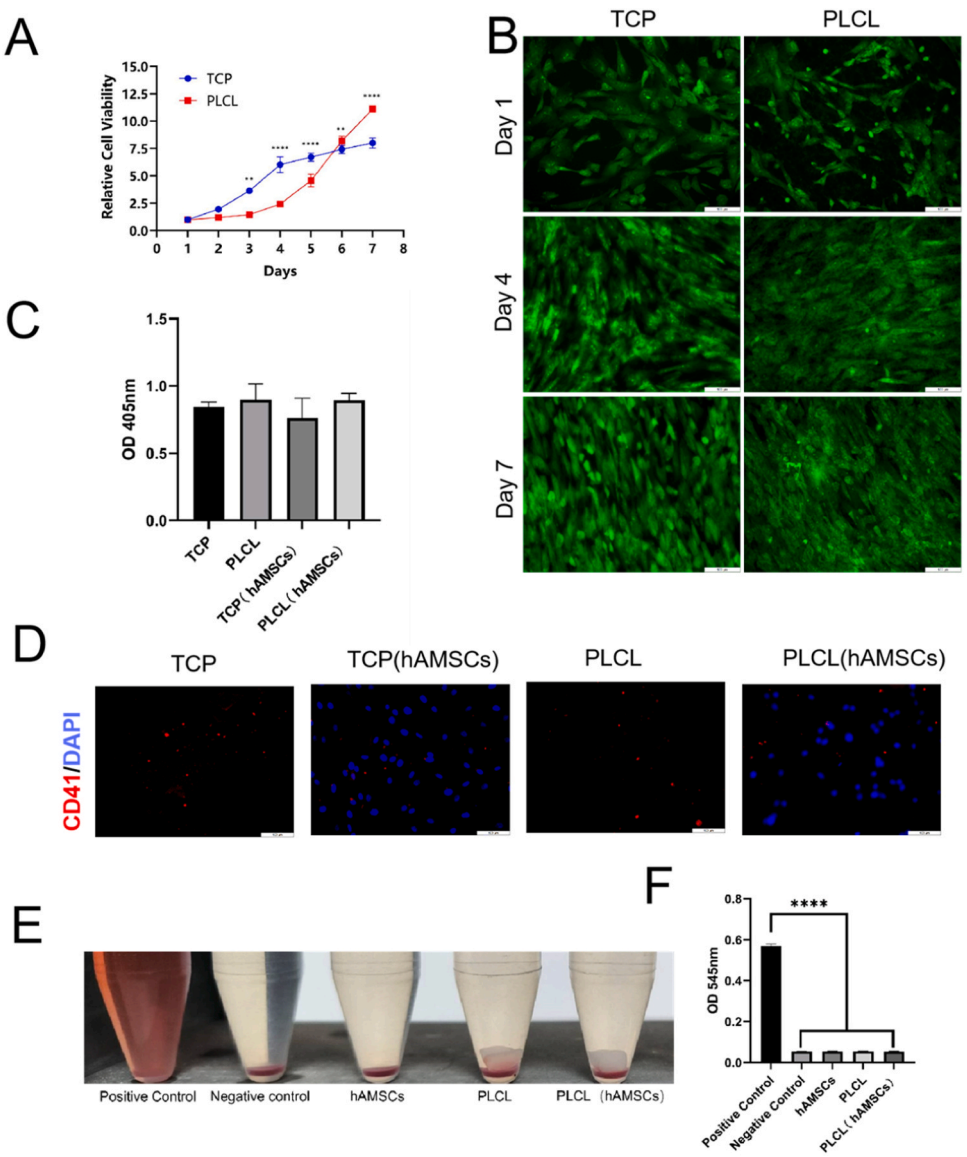

Fig. 3. Cell and blood campatability of the VG. A: Proliferation curve, B: Live/Dead staining, scale bar= 100 μm, C: thrombin activity, D: CD41 positive platelets on the VG under in vitro test, where TCP(hAMSCs) refers to hAMSC-seeded TCP surface, E：hemolysis image, where PLCL refers to vascular grafts without cells, hAMSCs refers to solely cells, and PLCL(hAMSCs) refers to vascular grafts with cells, F: qualitative measurement of hemolysis tests.

tissue compatibility. EVG staining showed that there were a large number of elastic fibers after loading hAMSCs (Fig. 5B). After 30 days, there was no calcium salt deposition in both groups (Fig. 5B). Safranin O staining showed that hAMSC loading group had more mucopolysaccharides and better biocompatibility (Fig. 5B). Immunofluorescence staining showed that compared with cell-free grafts, the hAMSC PLCL vascular grafts expressed much higher levels of endothelial markers CD31 and vWF, with almost complete endothelial coverage, which facilitated endothelialization and angiogenesis (Fig. 5C). The calculated endothelial coverage rate (the ratio of endothelial marker positive area to DAPI staining area) of hAMSC-seeding group was 87.63 ± 9.92 % (n = 3), which was significantly higher ($p < 0.01$) than that of cell-free group (20.03 ± 7.75 %, n = 3)(Fig. 5D). There was better regeneration of contractile SMCs in the lumen of hAMSC loading grafts (Fig. 5C), as indicated by the α-SMA-, calponin-, SM-MHC-positive cells, and also more synthetic SMCs (OPN-positive cells) were observed in hAMSC loading group. It is speculated that the synthetic type will gradually transit to the contractile type over time if remodeling time are longer. As indicated by CD68, both cell-free and cell-laden groups contained newly-recruited macrophages. Interestingly, the cell-free group recruited much more M1 subtype macrophages (iNOS-positive), while the cell-laden group recruited more M2 subtype (CD206-positive) (Fig. 5C). Collagen is a kind of crucial extracellular matrix protein in vascular wall. Collagen I and Collagen III positive areas clearly illustrate that the cell-laden group facilitated the extracellular matrix deposition (Fig. 5C). Lipase is the specific degrading enzymes of polyester polymers including PLCL. Both of the cell-free and cell-laden groups showed the existence of the lipase, but hAMSCs had no significant regulation on its expression level (Fig. 5C). Furthermore, hAMSCs could positively accelerate the extracellular matrix remodeling. This is supported by the fact that the cell-laden group expressed higher levels of MMP2 and MMP9 that eliminate the old extracellular matrices, and lower levels of TIMP1 and TIMP2 that inhibit the activity of MMP proteins (Fig. 5C). To track the distribution of human-derived cells after 30 days of remodeling, human nuclei specific antibody NUMA1 was employed. It was found that there were only a small number of hAMSCs or the derived cells in the vascular wall, suggesting most cells infiltrating the vascular wall are rat origin. It is also interesting to see that the distribution of human derived cells rearranged because some of them migrated to the outer side of the vascular wall (Fig. 5C). The collective results demonstrate

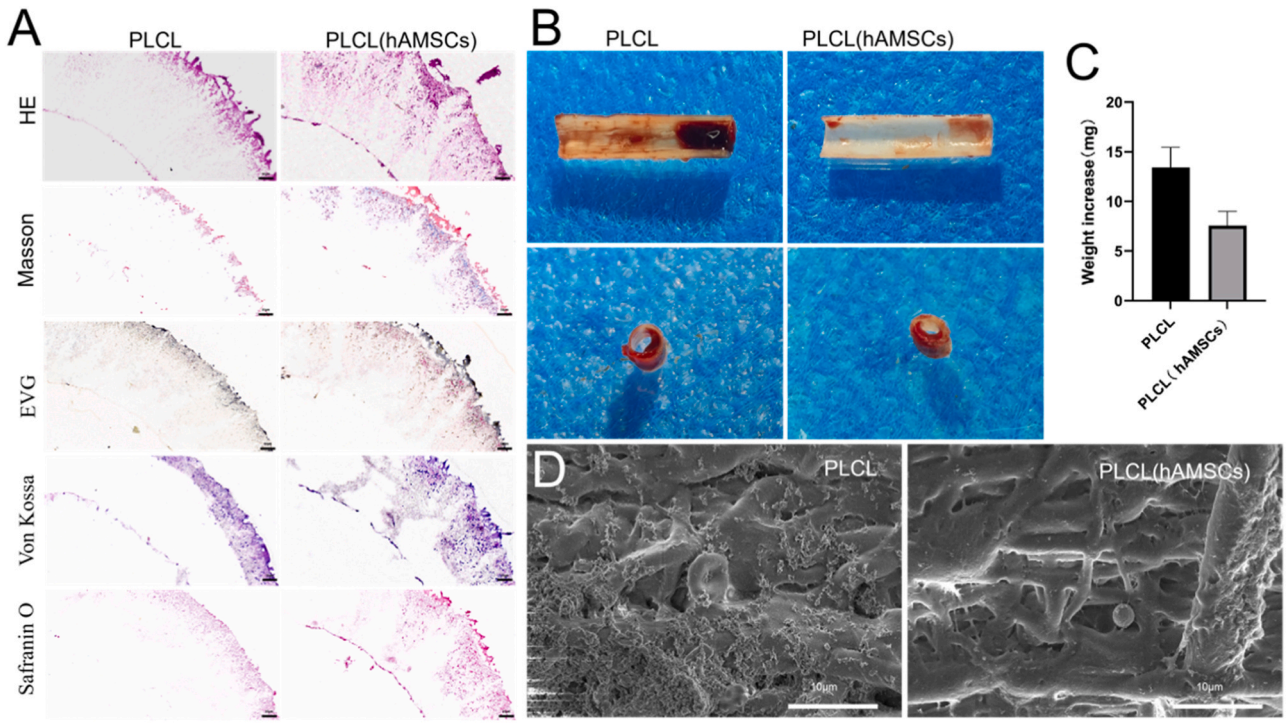

Fig. 4. Histological compatibility and acute anti-thrombosis capability of the VG. A: Histological staining after subcutaneous implantation for 2 weeks, scale bar= 50 μm, B: Gross observation of VGs after 2 h abdominal aorta replacement, C: weight increase in B, D: SEM image of VGs in B, scale bar= 10 μm.

that the hAMSC loading positively regulates the vascular graft remodeling via multiple pathways (Fig. 5C).

4. Discussion

SDVGs have always been a key challenge that urgently needs to be overcome in the field of cardiovascular disease treatment, and the preparation strategy based on stem cells and biodegradable electrospinning composites has opened up new avenues to overcome this challenge. This study selected the hAMSC, which is vibrant, abundant, easy to extract, non-invasive, and has no ethical issues, as a new type of seed cell. At the same time, the PLCL electrospinning scaffold, which has high biological safety, moderate mechanical properties, and strong regeneration guidance function, was selected as the scaffold substrate material to jointly construct a new vascular graft.

The feasibility of its transformation and application was verified in in in vitro and in vivo experiments. Firstly, this project successfully prepared an SDVG with uniform cell loading, length, inner diameter, wall thickness, spinning fiber diameter, etc. by loading hAMSC extracted. Subsequently, this study characterized the mechanical properties of hAMSC PLCL vascular grafts and found that they have good tensile strength, suture strength, and burst strength, making them suitable for blood vessel transplantation. The simulated suture experiment showed excellent surgical operability. PLCL electrospinning material does not affect the vitality of hAMSCs, demonstrating good cell compatibility. Experiments such as thrombin activity detection, hemolysis detection, and platelet adhesion detection have all demonstrated that hAMSC PLCL vascular grafts possess excellent blood compatibility.

The subcutaneous transplantation experiment in rats confirms the good tissue compatibility of hAMSC PLCL vascular grafts, and also preliminarily demonstrated the promoting effect of hAMSC on vascular regeneration. Acute thrombus testing shows that hAMSC PLCL vascular grafts have the ability to resist acute thrombosis, and the hAMSC plays a key role in it. The above series of experiments overall demonstrate the feasibility of conducting in vivo experiments on hAMSC PLCL vascular grafts.

Finally, this study implanted hAMSC-PLCL vascular grafts into a rat abdominal aorta replacement model for one month, and validated its in vivo patency and regenerative performance. The results showed that hAMSC PLCL vascular grafts achieved 100 % patency (5/5), while the patency rate of cell-free PLCL vascular grafts was only 60 % (3/5). This proves that hAMSC loading can improve the patency rate of PLCL electrospun vascular grafts, and also preliminarily verifies the feasibility of hAMSC PLCL vascular graft transformation application. Subsequent histological and immunofluorescent staining showed that hAMSC loading resulted in better regeneration of PLCL vascular grafts, including deeper cell infiltration, richer extracellular matrix deposition, and more complete intimal layer formation. At the same time, it also indicates that hAMSCs are highly likely to improve the patency and regeneration performance of vascular grafts by interacting with multiple targets. For example, hAMSC can reduce thrombus formation, promote accelerated endothelial repair, regulate macrophage polarization towards M2 direction, etc. However, the specific detailed mechanism still needs further exploration.

Although the research on hAMSC PLCL vascular grafts in this project has achieved some positive results, there are still many issues worth further exploration. Firstly, in the GLP level, more than 3 or 6 months follow-up and larger animal are used. However, the current research is based on small-scale rat experiments (5 per group) and short-term (1 month) in vivo evaluations, which actually limits the translation of the research. In order to better verify the reliability of our results and the feasibility of the vascular graft's translational potentials, it is necessary to observe the patency rate and regeneration effect of this vascular graft in larger scale animal experiments, longer-term in vivo implantation and larger animals such as pigs and dogs. Secondly, although it has been preliminarily proven that hAMSC can improve the patency and regeneration effect of vascular grafts through multi-target targeting, the existence of the main mechanism of action and whether there is interaction between each mechanism of action remains to be clarified.

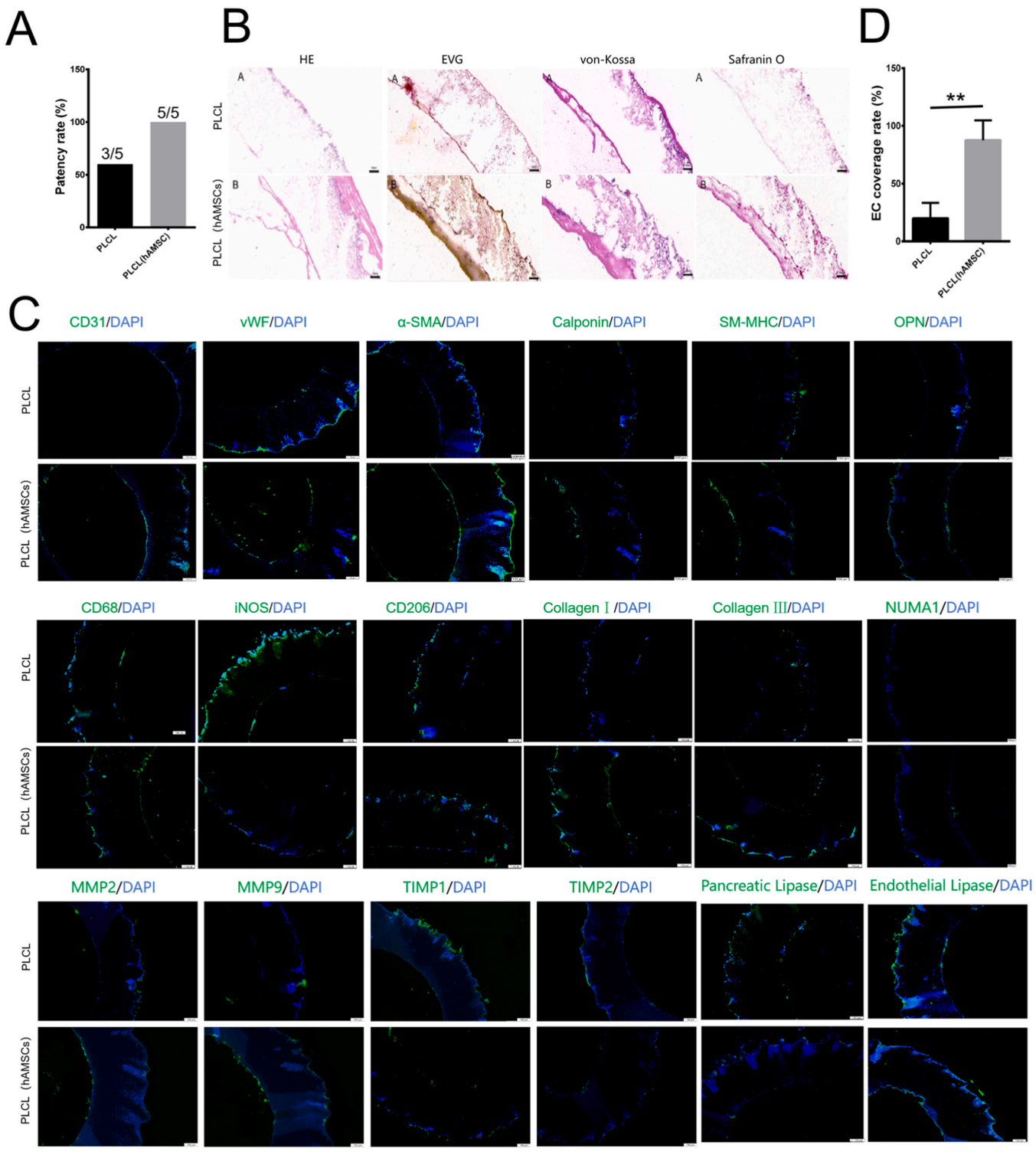

Fig. 5. In vivo patency and regeneration of the VG after 1 month implantation. A: Patency of the VG, B: Histochemical staining of the VG, scale bar= 50 μm, C: Immnunofluoresecnet staining of the VG, scale bar= 100 μm, D: Estimated EC coverage rate by endothelial marker positive area ratio, ** indicates $p < 0.01$, $n = 3$.

5. Conclusion

In the present study, a hAMSC-loading PLCL electrospun SDVG was successfully fabricated. The SDVG exhibited high mechanical strength, cyto-/hemato-/histo-campatibility, patency rate, and regeneration potential. The mechanism contributed to the fact that hAMSC loading reduced thrombus formation, increases endothelial coverage, smooth muscle cell recruitment (both contractile and synthetic type, and potentially promoting the transition of synthetic to contractile type), M1 to M2 macrophage shift, as well as extracellular matrix deposition and rearrangement (Fig. 6). The SDVG show promise for further pre-clinical tests and may offer new insights for the next generation SDVG development.

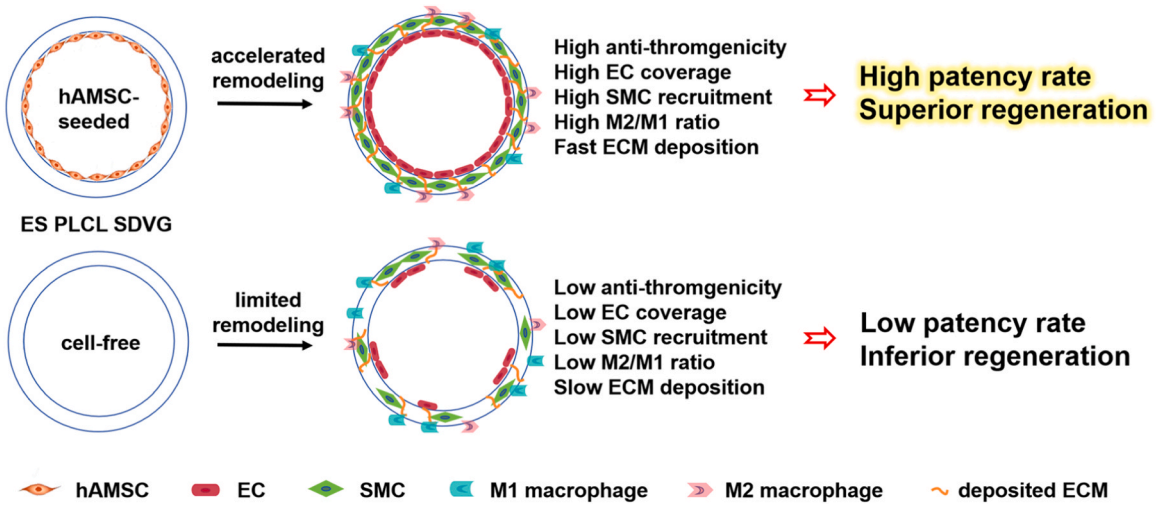

Fig. 6. The illustrated mechanism of action of hAMSC loading on electrospun (ES) PLCL vascular remodeling.

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CRediT authorship contribution statement

Wang Juan: Methodology, Investigation, Formal analysis, Data curation. **He Jing:** Methodology, Investigation, Formal analysis, Data curation. **Weng Dong:** Visualization, Methodology, Investigation, Data curation. **Wang Bin:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation. **Wang Nuoxin:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Wang Haoyuan:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **He Zhixu:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Mo Xiumei:** Writing – review & editing, Supervision, Resources, Investigation, Formal analysis, Data curation. **Wang Feng:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfb.2025.114559.

Data Availability

Data will be made available on request.

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