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Tailored endothelialization enabled by engineered endothelial cell vesicles accelerates remodeling of small-diameter vascular grafts

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ABSTRACT

Current gold standard for the replacement of small-diameter blood vessel (ID < 4 mm) is still to utilize the autologous vessels of patients due to the limitations of small-diameter vascular grafts (SDVG) on weak endothelialization, intimal hyperplasia and low patency. Herein, we create the SDVG with the tailored endothelialization by applying the engineered endothelial cell vesicles to camouflaging vascular grafts for the enhancement of vascular remodeling. The engineered endothelial cell vesicles were modified with azide groups (ECVs-N_3) through metabolic glycoengineering to precisely link the vascular graft made of PCL-DBCO via click chemistry, and thus fabricating ECVG (ECVs-N_3 modified SDVG), which assists inhibition of platelet adhesion and activation, promotion of ECs adhesion and enhancement of anti-inflammation. Furthermore, *In vivo* single-cell transcriptome analysis revealed that the proportion of ECs in the cell composition of ECVG surpassed that of PCL, and the tailored endothelialization enabled to convert endothelial cells (ECs) into some specific ECs clusters. One of the specific cluster, Endo_C5 cluster, was only detected in ECVG. Consequently, our study integrates the engineered membrane vesicles of ECVs-N_3 from native ECs for tailored endothelialization on SDVG by circumventing the limitations of living cells, and paves a new way to construct the alternative endothelialization in vessel remodeling following injury.

1. Introduction

Cardiovascular diseases (CVD), which cover a wide array of diseases from the cardiac muscle to the vascular system, stand as the leading cause of death globally [1–3]. Most of vascular diseases including atherosclerosis, thromboangiitis obliterans, traumatic arteriovenous fistula, and are highly associated with obstructive blood vessel [4–6]. Blood vessel replacement surgery proves to be an effective treatment option for the patients with severe cardiovascular diseases that normally cannot be fixed with interventional therapy [7]. Although some polymer materials such as polytetrafluorethylene (PTFE) and expanded PTFE (ePTFE) have achieved the clinical application on large-diameter vascular grafts (ID > 4 mm), small-diameter vascular grafts (SDVG) (ID < 4 mm) consisted of the aforementioned materials are still avoided due to their heightened risk of occlusion resulting from inadequate endothelialization, and there is no any product of SDVG in the current clinic so far [8,9]. It is therefore quite urgent to overcome the inaccessibility of foundation materials of SDVG.

Poor endothelialization is a crucial factor contributing to low patency rates in SDVG [10]. The endothelium, which acts as a natural barrier, prevents thrombosis and maintain vascular homeostasis by secreting bioactive factors like nitric oxide, prostacyclin, and heparan sulfate extracellular matrix [11–13]. Therefore, achieving instructive endothelialization in SDVG is essential to improve their patency rates [14]. Pre-seeding endothelial cells onto scaffold surfaces of SDVG *in vitro* can be regarded as a promising method to achieve rapid endothelialization. Nevertheless, the decrease in endothelial cell (ECs) activity after transplantation and the complex limitation of living ECs declines the

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effectiveness of this method [15]. Some biochemical cues on incorporating growth factors can partially enhance the migration and adhesion of ECs [16,17]. However, the modification of single growth factor or a fraction of functional molecules can hardly duplicate the entire role of ECs, with a plus of issues on controlled release and long-termed stability of biochemical factors [18]. Consequently, here we suggest a compromise proposal using endothelial cell membrane vehicles (ECVs) that is an alternative strategy between living ECs and single growth factor.

Alternative programs for endothelialization have great potential for improving patency rates and maintaining vascular homeostasis of SDVG after implantation [14]. Increasing evidences demonstrate the cell membrane vesicles can inherit most of functions of the source cells [19,20]. Similarly, the ECVs involve in recognition and transport of surface receptors, and participates in the metabolism and transportation of various substances and inhibits platelet adhesion and activation [21]. As a rapidly growing platform of biomaterial modification, the coating of cell membrane vesicles exhibits a vast potential for development, with its superior biocompatibility and various functions originated from source cells [19,22]. Cell membrane vesicles as for modifying biomaterials encompasses two main methods of chemical grafting and physical adsorption [20]. The former offers high stability by anchoring the cell membrane onto the material surface, but there is a potential risk regarding compromising the bioactivity of cell membrane. The latter achieves preservation of membrane activity by adsorbing it onto the material surface, but it may lead to burst release and instability due to weak linkage [23]. By adopting the preparation of cell membrane based on metabolic glycoengineering [24–26], a substantial number of azide groups can be introduced to the membrane surface and this enables non-destructive, precise and rapid linkage with DBCO-modified vascular grafts through click chemistry [27,28]. We assume that this innovative approach holds great promise for the modification of various bioactive cell membrane on biomaterials in terms of both stability and activity preservation.

In this study, the resulting surface editing of ECVs was modified with azide groups (ECVs-N_3) by the metabolic glycoengineering and proved to be firmly grafted onto the surface of SDVG through click reaction. We found the ECVs contained the multiple kinds of membrane proteins and simultaneously presented a mass of resemble functions of endothelial cells on the inhibition of platelet adhesion and activation and enhancement of anti-inflammation. *In vivo* evaluation of vascular replacement on the rabbit carotid confirmed that the ECVs-modified vascular grafts exhibited the rapid endothelialization and higher patency rate. Consequently, the creation of tailored endothelialization suggests the ECVs coating on the vascular grafts based on click chemistry might be a promising approach to advance vascular remodeling of SDVG.

2. Results

The expeditious establishment of a functional endothelial layer in SDVG is essential for its longevity and optimal physiological performance [29]. In this work, a pioneering approach for tailored endothelialization was employed to fabricate the intima of vascular grafts using the native endothelial cell vesicles (ECVs) for the purpose of minimizing the risk of thrombosis, rapid endothelialization, anti-inflammation and so on (Fig. 1). To prepare biomimetic intima in SDVG, the ECVs with azide groups (ECVs-N_3) were fabricated via three main steps, including metabolic glycoengineering, hypotonic and ultrasonic treatments

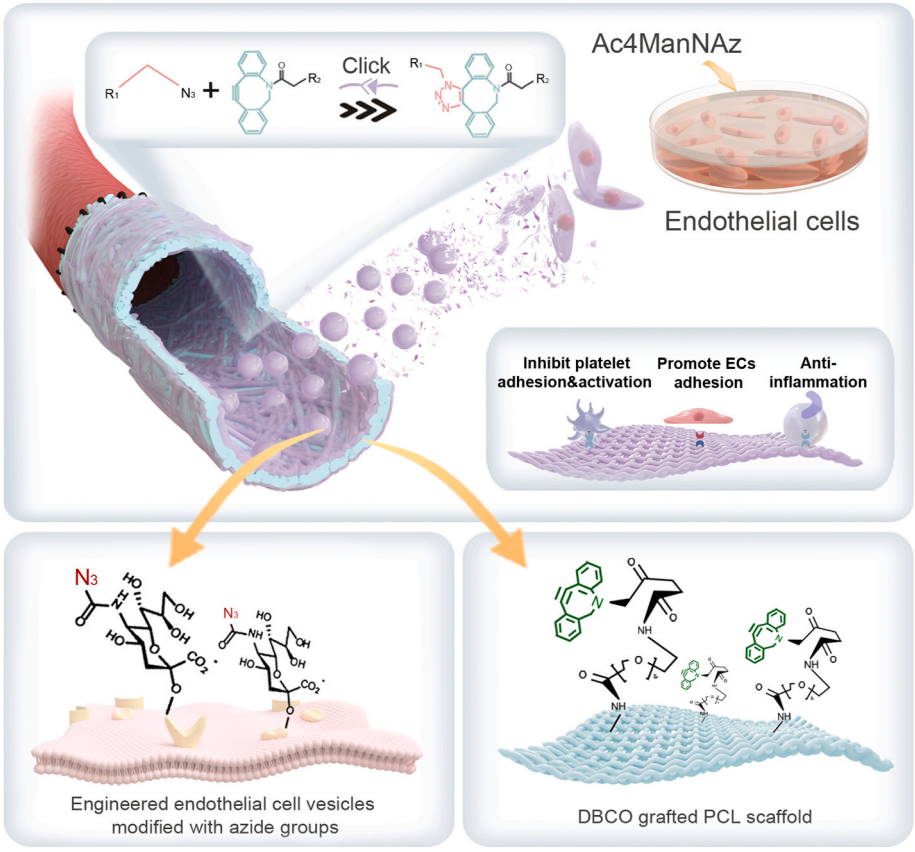

Fig. 1. Tailored endothelialization for small-diameter vascular grafts. To construct a specific tailored endothelialization on small-diameter vascular grafts (SDVG), the endothelial cells were modified with azide groups to fabricate the engineered endothelial cell vesicles (ECVs-N_3), and meanwhile the vascular grafts were made of PCL nanofiber linked with DBCO. Based on the click reaction between N_3 and DBCO, the ECVs-N_3 were grafted onto vascular grafts via a stable and covalent linkage. The SDVG modified with ECVs-N_3 were termed ECVG that presented a varies of functions on the inhibition of platelet adhesion and activation, promotion of ECs adhesion, and enhancement of anti-inflammation.

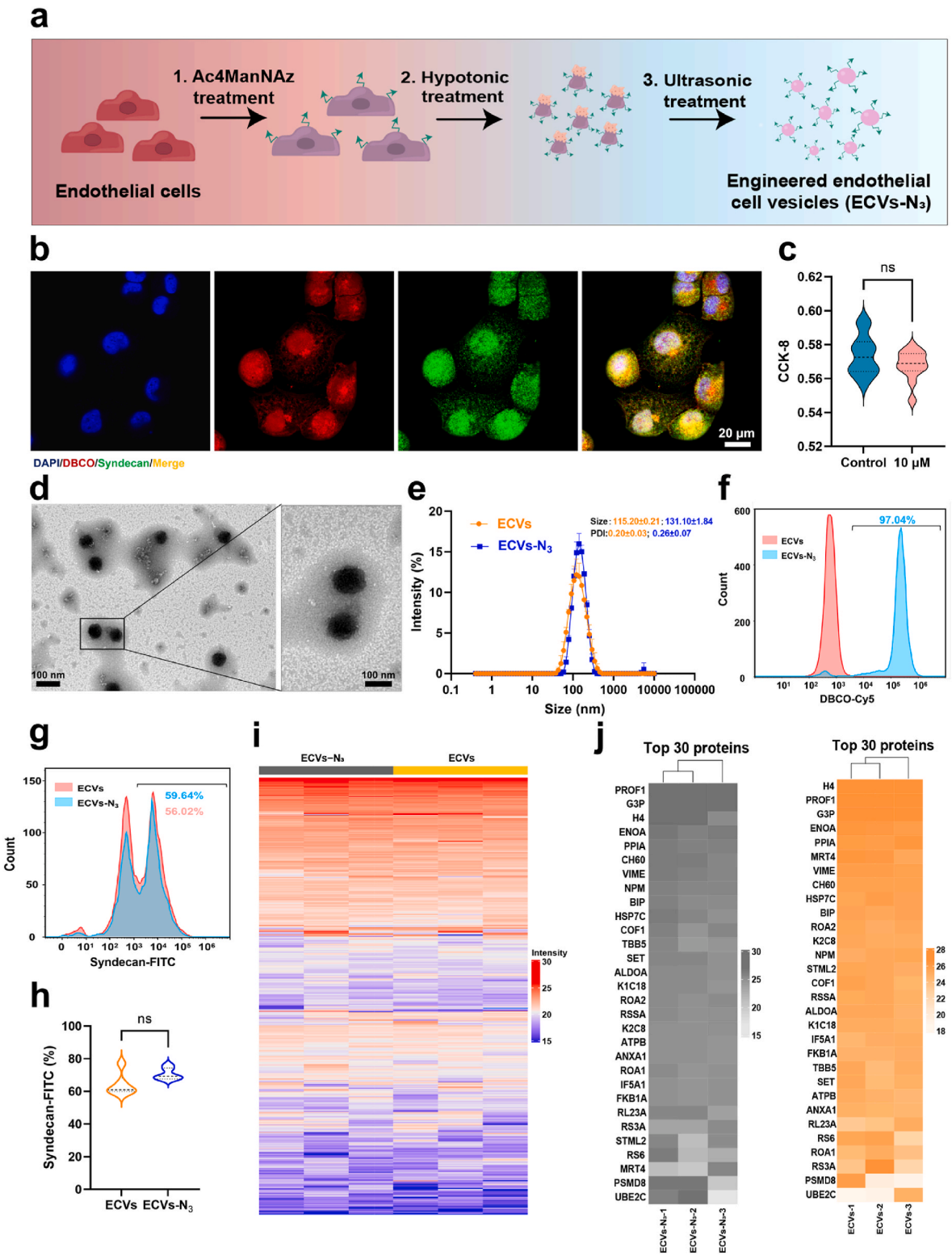

Fig. 2. Fabrication and identification of azide modified endothelial cell vesicles (ECVs-N_3). **(a)** Schematic illustration displayed the ECVs-N_3 fabrication through three steps including metabolic glycoengineering, hypotonic and ultrasonic treatments. **(b)** The azide modified ECs were identified using the fluorescent markers of DBCO-Cy5 and Syndecan-FITC. **(c)** Cell viability showed there was no significant influence on ECs after the treatment of 10 μm Ac_4ManNAz for 48 h. **(d)** Morphological size of ECVs-N_3 was about 100 nm by STEM. **(e)** DLS analysis indicated the hydrodynamic diameters of ECVs and ECVs-N_3 was about 100 nm. **(f)** Flow cytometry analysis verified that there was over 95 % of ECVs-N_3 modified with azide groups. **(g, h)** The syndecan was detected to be present in over 50 % of both ECVs-N_3 and ECVs. **(i)** Heatmap of the expression levels showed there were 943 proteins detected both in azide labeled and unlabeled ECVs. The log 10 expression values for the overlapping proteins were indicated by the colors shown in the scale. **(j)** Heatmap with the top 30 proteins showed the highly similar protein components between ECVs and ECVs-N_3.

(Fig. 2a). Firstly, the azide group was exogenously introduced onto the membrane surfaces of the ECs through the metabolic glycoengineering-mediated sialic acid pathway at 10 μM of N-azidoacetylmannosamine-tetraacetylated (Ac_4ManNAz). After 24 h, the fluorescence signal was exactly observed on the azide groups labeled ECs by laser scanning confocal microscopy (Fig. 2b). The treatment of Ac_4ManNAz showed no obvious effect on cell viability (Fig. 2c). ECVs and ECVs-N_3 were prepared through hypotonic, centrifugal and ultrasonic treatments. The primary elements of O, N, P were identified on both ECVs and ECVs-N_3 based on the elemental mappings (Fig. 2d–S1a and S1b). The average size of ECVs-N_3 and ECVs were about 131.10 nm and 115.20 nm, respectively. There was a little bit increase in size due to the modification of N_3. The zeta potential declined after the graft of N_3 (Fig. 2e and S2a). Flow cytometry analysis demonstrated there was nearly 97.05 % of ECVs-N_3 were positively labeled with Cy5 (Fig. 2f and S2b). Hence, there was no significant differences on zeta potential, size, morphology and element between ECVs and ECVs-N_3.

The syndecan chain of heparan sulfate was detected to be present in over 50 % of both ECVs-N_3 and ECVs (Fig. 2g and h). Heparan sulfate as an ancient family of transmembrane proteoglycans possesses the functions of promoting angiogenesis and inhibiting thrombosis. Proteomics identification displayed a total of 943 proteins were shared by the ECVs and ECVs-N_3 (Fig. S4). Heatmap showed there was no significant difference of the expression levels of the 943 proteins between ECVs and ECVs-N_3 (Fig. 2i). Moreover, using the top 30 proteins as a reference, we confirmed the highly consistency of ECVs and ECVs-N_3 on the protein components (Fig. 2j). Based on above analysis of protein components within ECVs and ECVs-N_3, we found there was a high degree of consistency between ECVs and ECVs-N_3, implying that most of protein components were not changed during the Ac_4ManNAz treatment (Fig. S3 and S4).

Small-diameter vascular grafts (SDVG) modified with the ECVs, termed ECVG, were fabricated through three steps of chemical modification processes based on click chemistry (Fig. 3a, b and S5a). Firstly, the electrospun polycaprolactone (PCL) grafts were modified with positive charged amine group (-NH_2) by 1,6-hexanediamine. The ninhydrin coloration assay indicated there was an obvious change in color before and after the coating of amine group on the PCL scaffolds (Fig. 3c). NHS-PEG_4-DBCO group was subsequently introduced to the NH_2-PCL grafts to achieve the DBCO grafted PCL (DBCO-PCL) linkage that presented a strong green fluorescent signal stained with FITC-PEG-N_3 (Fig. 3d). Lastly, DBCO-PCL scaffolds were co-incubated with ECVs-N_3 solution for 4 h at 37 °C. The ECVs-N_3 were effectively and precisely incorporated onto DBCO-PCL scaffolds, leading to the formation of a micron-scale vesicle coating through coverage, as confirmed by specific fluorescence staining analysis, SEM observation, and elemental mapping (Fig. 3e–3f, S6 and S7). SDS-PAGE results revealed a high level of consistency between ECVs-N_3 and ECVG in terms of their protein bands, indicating that the majority of membrane proteins was preserved throughout the fabrication processes (Fig. 3g). Heparan sulfate derived from ECs, as a key role of anti-coagulant ingredients, was detected on the ECVG (Fig. 3h). Covalent bonding of ECVs-N_3 onto PCL scaffolds largely contributed the ECVs content and stability (Fig. 3i). The release kinetics of ECVs-N_3 showed that there was still about 80 % of ECVs on the ECVG in a shaking condition for 10 days. In contrast, ECVs were physically absorbed in PCL scaffolds, there was only 10 % of ECVs with the same processing, implying the higher stability for the covalent modification. (Fig. 3j and Fig. S8). There was no significant change of tensile strength for vascular grafts before and after modification (Fig. 3k and 3l). All these results indicate that the tailored endothelialization of vascular grafts have been successfully constructed.

Evaluation of cell adhesion confirmed that the well-designed ECVG could support more adhesion of ECs (Fig. 4a and b). Although there was no difference between ECVG and PCL groups in ECs proliferation with the extend in culture time (Fig. 4c). Therefore, the interaction between vascular cells and ECVG clearly indicated that ECVs modification contribute to cell adhesion, simultaneously exhibiting a potential for inducing rapid self-endothelialization. Platelet adhesion and activation are highly associated with the physicochemical properties of substrate surface. ECVG stained with mepacrine exhibited the excellent anti-adhesion to platelets, and fewer platelets were able to be adhered to the inner surface of the ECVG than that in the PCL grafts (Fig. 4d and 4e). CD62p staining showed the minimal platelet activation on ECVG (Fig. 4d and 4f). PCL and ECVG exhibited hemolysis rates below 5 % (Figs. S5b and S5c), adhering to international (ISO 10933-4) and national (GB/T 16886.4) standards. These findings suggest that the tailored endothelialization contributes to the enhancement of ECs adhesion and inhibition of platelet adhesion and activation.

To evaluate the *in vivo* biocompatibility and tissue regenerative capability of scaffolds, three circular pieces (d = 2 cm) of ECVG and PCL grafts were implanted underneath the dorsal skin of rats (Fig. 4g). 7 days of post-implantation, the scaffolds were collected and analyzed using hematoxylin and eosin (H&E) and Masson staining. Both ECVG and PCL grafts were encapsulated by regenerative tissues, indicating stimulation of the host immune response (Fig. 4h and i). Immunofluorescence images revealed that ECVG increased the protein expression of Arg1 while decreased the expression of iNOS compared with PCL grafts (Fig. 4j–m), implying ECVG triggering the anti-inflammation reaction. Further studies were conducted to investigate whether ECVs-N_3 modification could promote vascularization. Immunofluorescence analysis of CD31 showed that more blood vessels formed around ECVG than PCL grafts, suggesting that ECVG have the potential to promote vascularization (Fig. 4n and o). Overall, these results suggest that the tailored endothelialization endows vascular grafts with superior biocompatibility and tissue regeneration capability.

To explore further *in situ* performance of ECVG, the vascular replacement model of the rabbit carotid artery was used to evaluate ECVG and PCL grafts (Fig. 5a and b). After 3 months, the patency rates of ECVG was 83.3 %, which was significantly higher than that of PCL (66.7 %) (Fig. 5c and d). All vascular grafts were explanted after 3 months. Optical images showed there was no blood clots within grafts (Fig. 5e). HE staining showed the regenerative vascular tissues were continuous in both ECVG and PCL groups, and notably the thickness of neo-vessel and luminal diameter of ECVG were close to native vessels, which indicated the neo-vessel with natural vascular structure and scale was formed (Fig. 5f and 5g). EVG staining indicated that small amounts of elastic fibers of vascular ECM successfully regenerated in both grafts (Fig. 5h). Immunofluorescence staining of CD31 revealed a round monolayer of endothelium on the luminal surface 3 months of post-implantation, and the coverage ratio of ECVG was superior to PCL group (Fig. 5i and 5j). Both ECVG and PCL grafts presented the smooth muscle regeneration, as characterized by the positive staining of α-SMA (Fig. 5k and 5l). Alizarin red staining demonstrated there was some obvious calcium nodule in regenerative blood wall of PCL, but not in ECVG (Fig. 5m). Immunofluorescence images revealed that ECVG increased the protein expression of CD206 while decreased the expression of iNOS compared with PCL grafts (Fig. S9), implying that ECVG exhibit a less intense inflammatory reaction. In summary, these findings illustrate that ECVG effectively enhance vascular remodeling and sustained patency ascribed to its tailored endothelialization.

To investigate the role of ECVs in vascular remodeling post-implantation, the single-cell transcriptome analysis was conducted, capturing a total of 8388 cells in PCL and 6897 cells in ECVG. Following cell filtering, 14,045 single-cell transcriptomes were integrated into the final dataset (7687 cells in PCL and 6358 cells in ECVG). According to uniform manifold approximation and projection (UMAP) analysis, the cells were categorized into monocytic cells (clusters 0, 1, 3, 7 and 11), ECs (cluster 8 and 14), pericytes (cluster 16), T&B cells (cluster 6 and 10), fibroblasts & myofibroblasts (clusters 2, 4, 5 and 17), neutrophils (cluster 9) and plasma cell (cluster 12) (Fig. 6a–S10 and S11). In scRNA-seq profiles, cell types were defined according to the genetic expression (Fig. 6b). One of them, the proportion of ECs in the cell composition of

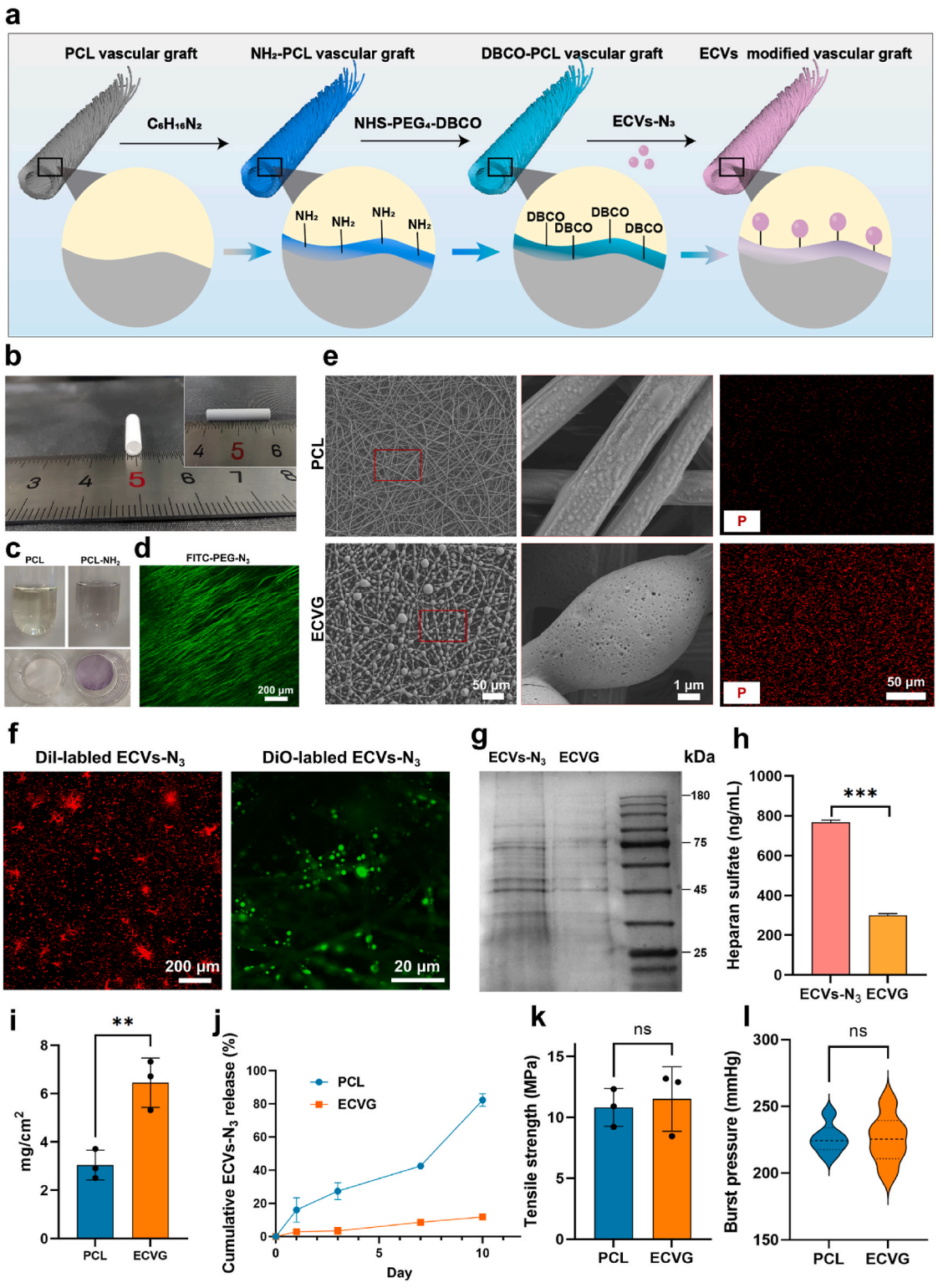

Fig. 3. Fabrication and characterization of ECVG. **(a)** The electrospun PCL vascular graft was firstly modified with positive charged amine group (-NH_2) by 1,6-hexanediamine. The NHS-PEG_4-DBCO group was subsequently connected to the NH_2-PCL grafts to achieve the DBCO-PCL vascular graft, which was covalently linked with ECVs-N_3 via click chemistry. **(b)** Optical images showed the ECVG belonged to small-diameter with about 2 mm in diameter and 2 cm in length. **(c)** The amine groups were comparably analyzed by ninhydrin coloration in both PCL and NH_2-PCL scaffolds. **(d)** The PCL scaffold with DBCO group was labeled by FITC-PEG-N_3. **(e)** There was obvious morphological change of the vascular grafts before and after ECVs-N_3 modification, and specific element (Phosphorus) of ECVs-N_3 was verified via EDS mapping. **(f)** DiI (red) or DiO (green) labeled ECVs-N_3 were present on the surface of ECVG. **(g)** SDS-PAGE results indicated that there was a high degree of consistency between ECVs-N_3 and ECVG, implying that most of membrane proteins were retained. **(h)** Heparan sulfate of endothelial cell membrane was present on the ECVG, implying its potential on anti-coagulation. **(i)** The comparison of protein content on the different scaffolds indicated that the covalent bonding of ECVs-N_3 onto ECVG largely contributed the ECVs content and stability compared to physical absorption. **(j)** The cumulative release of ECVs-N_3 from ECVG demonstrated that the click reaction significantly enhanced the stability of ECVs modification. **(k and l)** Results of tensile strength and burst pressure showed the process of modification had no obvious influence on the mechanical strength of vascular grafts.

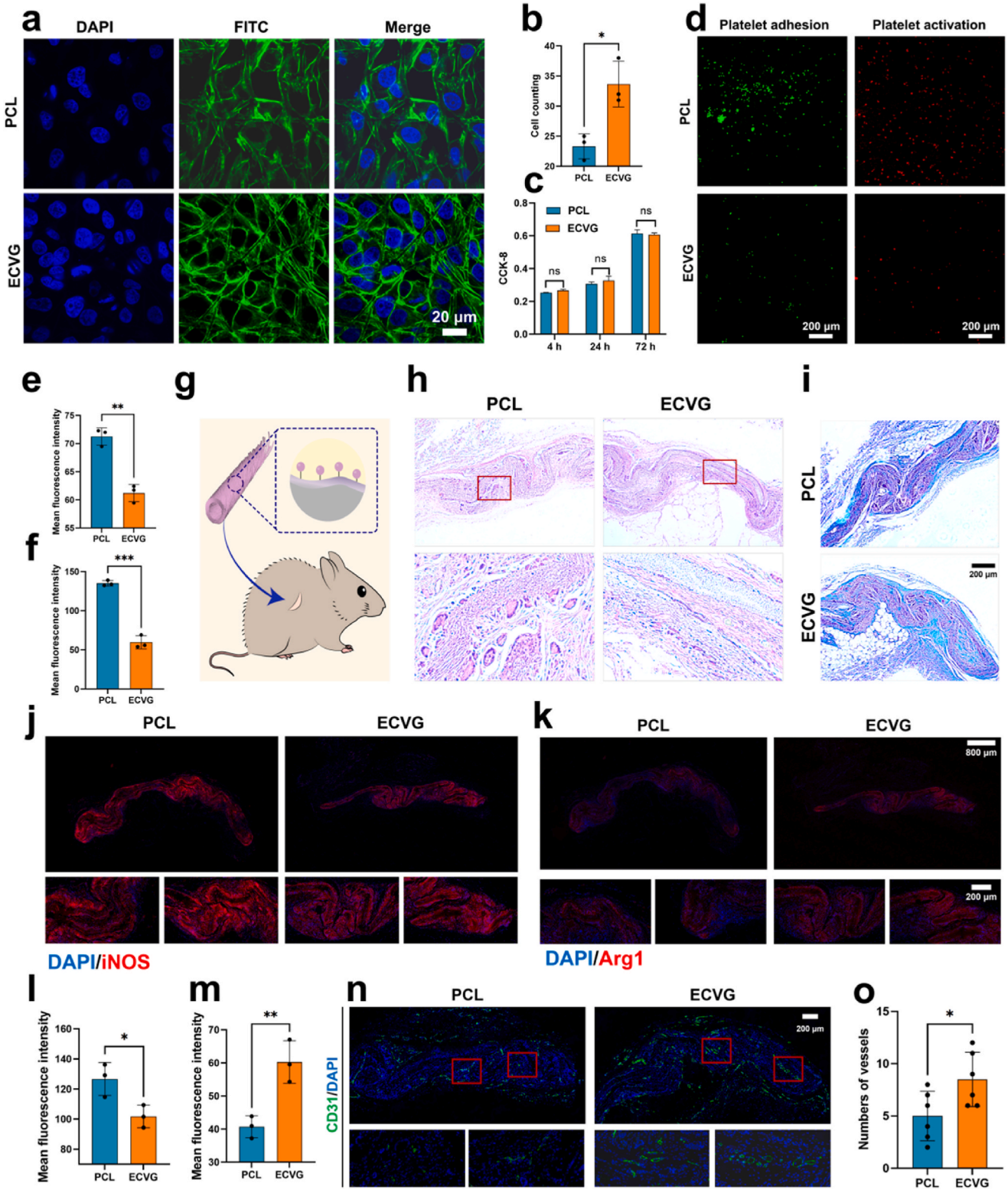

Fig. 4. Biocompatible and anti-inflammatory evaluation of ECVG. **(a)** Both ECVG and PCL scaffolds were able to well support the adhesion of endothelial cells (ECs). **(b)** Cell counting based on three fluorescence images demonstrated cell adhesion was elevated based on ECVs modification. **(c)** There was no significant difference on cell proliferation between ECVG and PCL scaffolds. **(d)** The ECVs modification was beneficial to the inhibition of platelet adhesion and activation. **(e and f)** The mean fluorescence intensity of platelet adhesion and activation was quantified, respectively. **(g–i)** According to evaluation of subcutaneous implantation *in vivo*, H&E and Masson staining showed both ECVG and PCL could fast induce the newly-formed tissue and the obviously collagen deposition was detected 7 days after implantation. **(j, l)** Representative immunofluorescence staining and the corresponding quantification for the inflammation marker of iNOS showed the inflammation reaction in PCL group was higher than that in ECVG. **(k, m)** Representative immunofluorescence images and the corresponding quantification for anti-inflammation marker of Arg1 verified the consistent results, indicating that the ECVG could significantly suppress the inflammation reaction. **(n, o)** Representative fluorescence staining and related quantification for numbers of vessel demonstrated the ECVG promoted vascularization around the scaffolds.

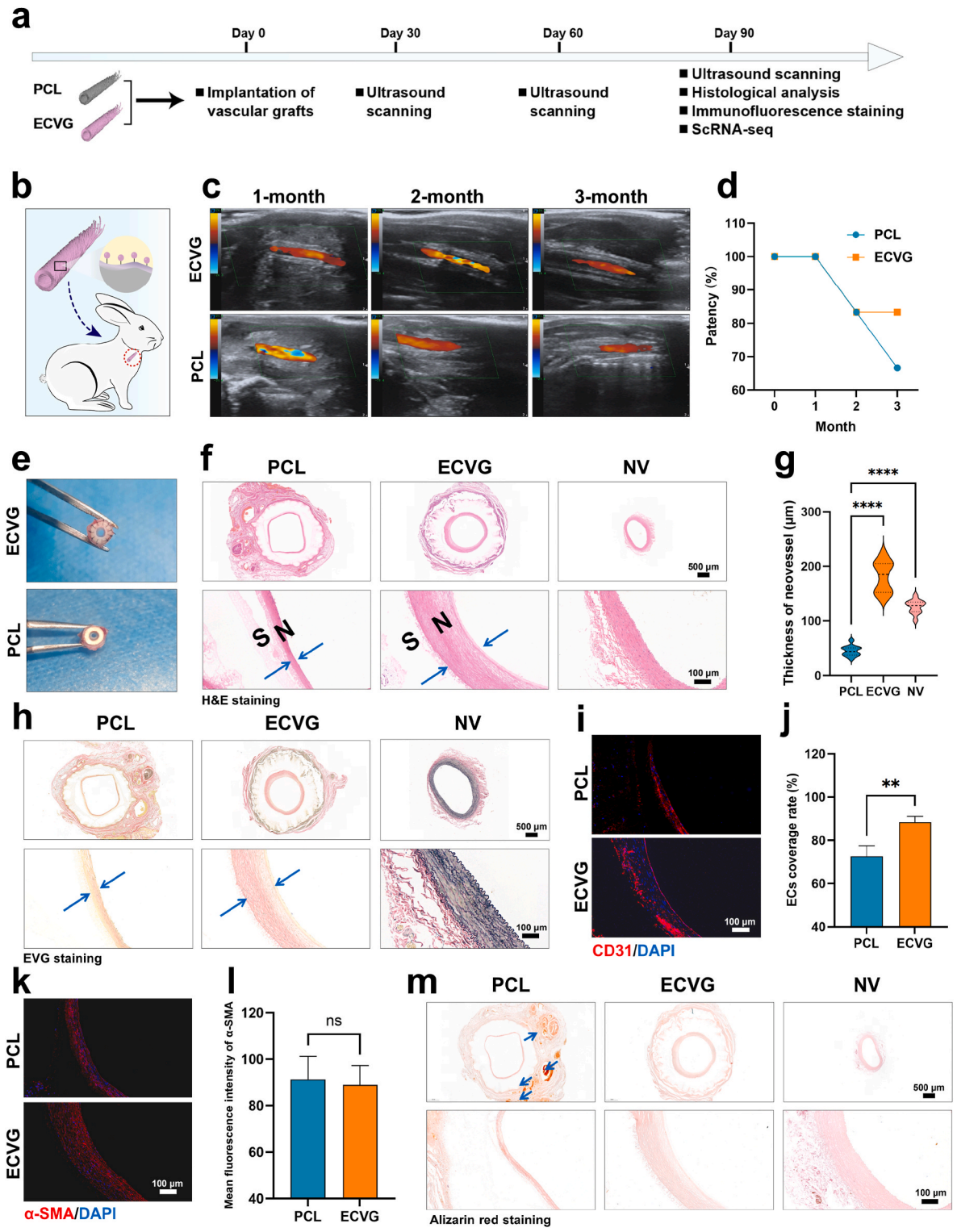

Fig. 5. ***In vivo*** **evaluation of ECVG on the rabbit carotid artery model. (a)** A workflow showed the evaluation outline for neo-vessel induced by ECVG. **(b)** Schematic diagram showed the *in vivo* implantation of ECVG into the carotid artery of the rabbit. **(c and d)** Doppler ultrasound monitoring of vascular grafts evaluated the patency rates on the different timepoints. The patency rates of ECVG was about 83.3 %, and significantly higher than 66.7 % of PCL groups. **(e)** Optical images of explanted vascular grafts showed there was no blood clots in circular lumens of ECVG and PCL 3 months after implantation. **(f)** H&E staining displayed that both ECVG and PCL were able to induce the neo-intima formation (N: new tissue, S: Scaffold). **(g)** The thickness analysis of neo-intima verified the ECVG could significantly promote intimal regeneration and very close to native artery (NA). **(h)** EVG staining indicated that elastic fibers form in very small amounts in the regenerated tissue in both grafts. **(i and j)** The regenerative endothelium quantified by coverage of $CD31^{+}$ cells indicated ECVG promoted endothelialization *in vivo*. **(k and l)** The infiltration of vascular smooth muscle cells was further stained by anti-α-SMA, which verified the tunica remodeling in both grafts. **(m)** Alizarin red staining analysis showed that there was obvious calcification around PCL grafts.

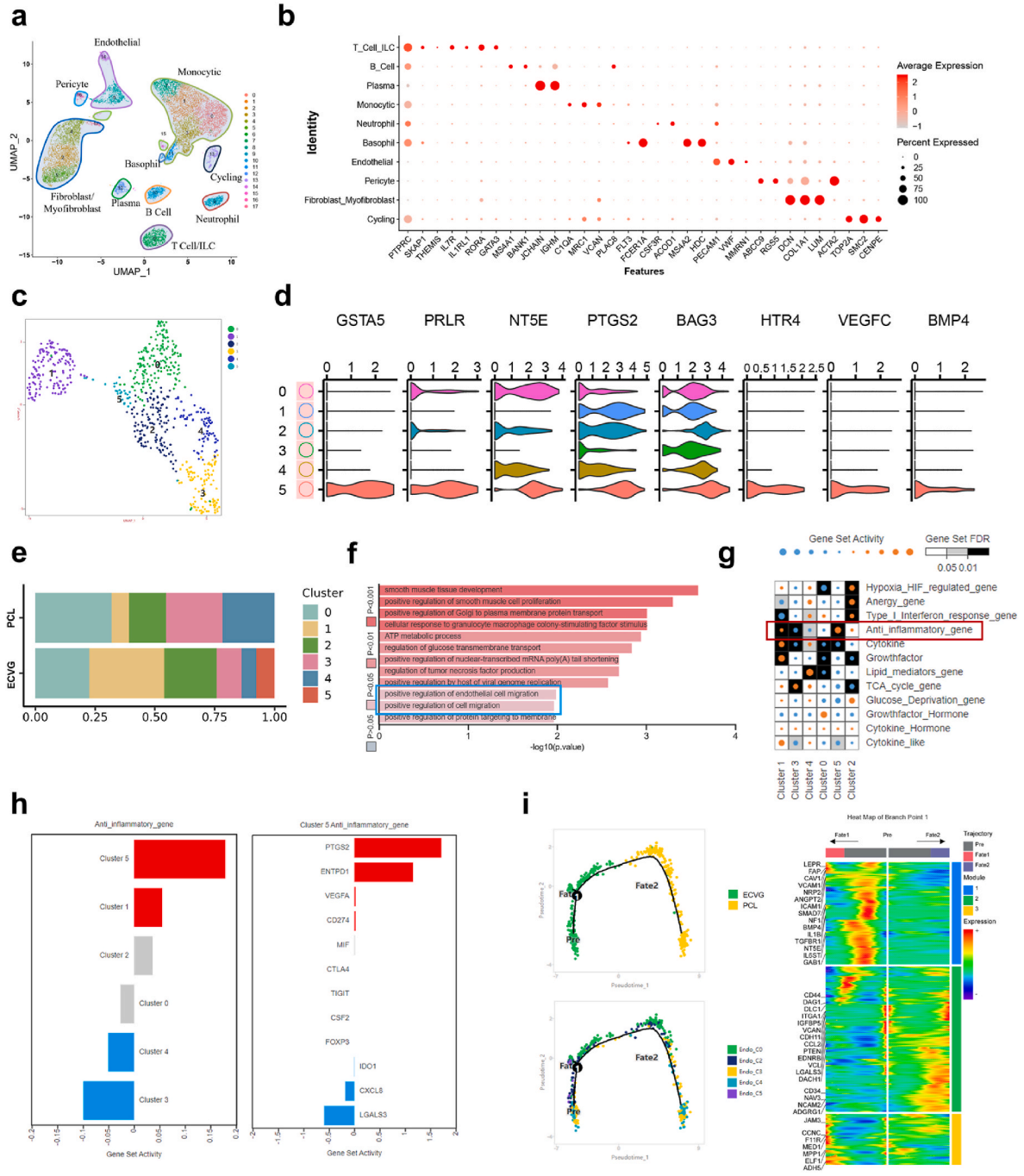

Fig. 6. The single-cell atlas of the tailored endothelialization-mediated microenvironment. **(a)** Overview of cell clusters identified in the composite scRNA-seq dataset. **(b)** Dot plots showed cell types based on the marker genes. **(c)** The defined ECs were subdivided into six clusters of 0, 1, 2, 3, 4, 5 (Endo_C0-C5). **(d)** Violin plots showed the marker gene expression in Endo_C5 across the other clusters and ECVG showed the specific Endo_C5. **(e)** The different subclusters of ECs were present in the composition of ECVG and PCL, and Endo_C5 was only found in ECVG. **(f)** Marker GO analysis of Endo_C5. **(g and h)** QuSAGE analysis showed gene set activity of different clusters and Endo_C5 enriched anti-inflammatory genes. **(i)** Analysis of the differentiation trajectory displayed a featured change of cell fate dominated by ECVG and PCL, as well as the origin and end point of five EC clusters according to the expression changes of characteristic genes.

ECVG surpassed that of PCL (Fig. S12). The defined ECs were subdivided into six clusters: 0, 1, 2, 3, 4 and 5 (Endo_C0-C5) in UMAP analysis (Fig. 6c). Among them, Endo_C5 was characterized by a specific cluster exclusively existed in ECVG (Fig. 6d–e). Gene Ontology (GO) analysis suggested that Endo_C5 was implicated in the positive regulation of ECs migration and differentiation, as well as the control for tissue development of smooth muscle cells (Fig. 6f). Quantitative set analysis of gene expression (QuSAGE) was employed to figure out the roles of different clusters in vascular remodeling. The results revealed that Endo_C5 exhibited an expression advantage of anti-inflammatory genes (Fig. 6g and 6h). Pseudo-time analysis of EC clusters unveiled 2 kinds of cell fates and EC cluster in a pre-state transitioning into fate 2 occupied by Endo_C5 via gene expression during the vascular regeneration in ECVG (Fig. 6i). In general, the single-cell transcriptome analysis further demonstrates that the tailored endothelialization plays a central role in endothelial cell differentiation and migration during post-implantation vascular remodeling, and meanwhile promotes the angiogenesis, as well as anti-inflammatory.

3. Discussion

Homogeneous and rapid endothelialization is of utmost importance for small-diameter vascular grafts (SDVG) to maintain vascular homeostasis involving angiogenesis, thrombosis inhibition, intimal hyperplasia and anti-inflammation [30,31]. Pre-seeding living endothelial cells (ECs) onto SDVG in static, dynamic or bioreactor seeding ways, was considered as a promising strategy, which enhanced the biocompatibility, reduced thrombogenicity and increased durability and functional lifespan of the graft to mimic the natural ECs of vessels [32]. However, obtaining a sufficient number of viable endothelial cells for seeding, while ensuring the mechanical integrity of the graft, and meeting regulatory standards for clinical application are significant challenges in advancing endothelial cell-seeded SDVGs [33]. In the end, it did not become the mainstream method for improving SDVG. Thus, herein we introduce a middle way of tailored endothelialization with engineered EC membrane vesicles in place of ECs to be fixed onto intimal surface for constructing a biomimetic vascular graft of ECVG. This kind of vascular graft might make a balance effect between cell seeding and molecular modification for improving vascular patency rates and maintain vascular homeostasis. The resulting ECVs modification of alternative endothelialization with the potential advantages of high yield, low cost and storage convenience can substitute living ECs to fabricate the vascular intima of SDVG.

The native intima of blood vessels contributes the various functions on the inhibition of platelet adhesion and activation, promotion of ECs adhesion and enhancement of anti-inflammation [34–36]. Achievements of current ECVG with the above broad functions are probably benefit from the highly similarity between ECVs and ECs membrane. For instance, heparan sulfate as a critical membrane glycosaminoglycan has a lot of functions on vascular remodeling and inflammatory regulation [37,38]. Both ECVs and ECs can possess the bioactive heparan sulfate to present the regulation potential on vascular homeostasis. Also, the single-cell transcriptome confirms the regulation mechanism of ECVs is to promote ECs migration and differentiation as well as generating the specific clusters of ECs subset in vascular remodeling. Thus, our current study provides a promising method of alternative endothelialization using ECVs to replace ECs. Bioactive membranes not only inherit most properties of donor cells, but also overcome many drawbacks of issues using living cells.

There is a certain contradiction between stable modification and activity retention, especially in a complex blood flow environment. The chemical grafting method provides high stability by firmly attaching the cell membranes to the material surface, but there is a potential risk of compromising the membrane's bioactivity [39]. Conversely, the physical adsorption method maintains membrane activity by adsorbing it onto the material surface, but it may result in a burst release of cell membranes [39]. Click chemistry is highly selective, efficient, and reliable and widely used for molecular fragment joining between cells and biomolecules [40], which enables mild reactions, preserving biomolecule integrity, facilitating specific attachment without cellular disruption, versatile conjugation across biomolecules, precise control over attachment, and compatibility with diverse modifications for multifunctional biomaterial development in biomedicine. But click chemistry depends on the presence of appropriate functional groups on biomolecules, which requires pre-processing of biologically active molecular materials and increase complexity and cost compared to simpler conjugation methods [41]. Overall, click chemistry is biorthogonal and can be performed in complex biological environments like living cells, especially suitable for the modification of bioinert materials by bioactive factors. In this study, we apply a specific metabolic glycoengineering and click chemistry strategy that can make a stable graft of ECVs onto the vascular grafts based on the crosslink of azide and DBCO. Our findings verify that the most of ECVs-N_3 can make a stable existence in a simulating shaking condition. On the contrary, the physical absorption of ECVs can only retain 10 % ECVs under the same processing. Moreover. the engineered ECVs modified with azide groups present similar sizes, zeta potentials, morphologies and protein components with ECVs-N_3 as well as physiological functions of EC membrane. Therefore, our current approach constructs SDVG with the dual functional integration between stability and bioactivity.

Up to now, there is still no any commercially products of SDVG in clinic [8]. Increasing evidences indicate that the breakthrough of SDVG might be largely dependent on the innovation of foundation material [42]. Despite current bioinert and non-biodegradable polymers containing PTFE and ePTFE receive great success in large-diameter vascular grafts, they are not instructive to obtain SDVG due to the huge difference on hemodynamics between small and large vessels [43]. Thus, our current work attempts to utilize the FDA-approved and biodegradable polymer of PCL as a foundation material to fabricate SDVG. This kind of biodegradable vascular grafts can induce the generation of neo-vessel, and is eventually replaced by the regenerative and native blood vessel and acquire a long-term and even forever patency in a small-diameter vascular graft.

4. Conclusion

Highly bionic vascular intima generated from the engineered endothelial cell vesicles (ECVs) plays a pivotal role in promoting endothelial cell adhesion and migration, and inhibiting platelet adhesion and activation via carrying natural and intact membrane proteins. This biomimetic coating of ECVs provides an effective method for the tailored endothelization modification of vascular grafts, with the advantages of coating stability and preservation of biological activity due to metabolic glycoengineering and click chemistry. The resultant ECVG presents to tremendously elevate endothelization and enhance long-term patency based on the rabbit carotid artery model *in vivo*. The single-cell transcriptome analysis further verifies that the ECVs play a crucial role in endothelial cell migration and differentiation, angiogenesis, and anti-inflammation during post-implantation vascular remodeling. In consequence, the tailored endothelialization based on ECVs camouflage may offer a promising approach to improve vascular remodeling with similar functions of living ECs, and also presents a huge potential application in other injured tissue remodeling.

Data availability statement

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Ethics approval and consent to participate

All animal procedures were carried out in accordance with the protocol approved by the Institutional Animal Care and Use Committee of HUST.

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.

CRediT authorship contribution statement

Zihao Wang: Writing – original draft, Project administration, Methodology, Investigation. **Mengxue Zhou:** Writing – original draft, Methodology, Investigation. **Mengyu Li:** Methodology, Investigation. **Jinyu Li:** Methodology, Investigation. **Shengmin Zhang:** Supervision, Project administration. **Jianglin Wang:** Writing – original draft, Supervision, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2024.07.006.

References

[1] F.D. Fuchs, P.K. Whelton, High blood pressure and cardiovascular disease, Hypertension 75 (2) (2020) 285–292.
[2] M.E. Walker, R. De Matteis, M. Perretti, J. Dalli, Resolvin t4 enhances macrophage cholesterol efflux to reduce vascular disease, Nat. Commun. 15 (1) (2024) 975.
[3] Y. Shen, J. Cui, X. Yu, J. Song, P. Cai, W. Guo, et al., Recent advances in three-dimensional printing in cardiovascular devices: Bench and bedside applications, Smart Materials in Medicine 5 (1) (2024) 36–51.
[4] Q. Chen, J. Chen, J. Li, Y. Cheng, R. Zhang, Z. Liu, Recent advances of oxidative stress in thromboangiitis obliterans: biomolecular mechanisms, biomarkers, sources and clinical applications, Thromb. Res. 230 (2023) 64–73.
[5] N.J. Leeper, N.M. Hamburg, Peripheral vascular disease in 2021, Circ. Res. 128 (12) (2021) 1803–1804.
[6] L. a D. Ottewell, M.P. Paranathala, C.H. Robson, A.J. Jenkins, Traumatic arteriovenous fistula from internal maxillary artery following trigeminal balloon compression, Br. J. Neurosurg. (2023) 1–4.
[7] F.M. Zimmermann, V.Y. Ding, N.H.J. Pijls, Z. Piroth, A.H.M. Van Straten, L. Szekely, et al., Fractional flow reserve-guided pci or coronary bypass surgery for 3-vessel coronary artery disease: 3-year follow-up of the fame 3 trial, Circulation 148 (12) (2023) 950–958.
[8] N. Wang, H. Wang, D. Weng, Y. Wang, L. Yu, F. Wang, et al., Nanomaterials for small diameter vascular grafts: overview and outlook, Nanoscale Adv. 5 (24) (2023) 6751–6767.
[9] H.H. Greco Song, R.T. Rumma, C.K. Ozaki, E.R. Edelman, C.S. Chen, Vascular tissue engineering: progress, challenges, and clinical promise, Cell Stem Cell 22 (4) (2018) 608.
[10] H. Ding, X. Hou, Z. Gao, Y. Guo, B. Liao, J. Wan, Challenges and strategies for endothelializing decellularized small-diameter tissue-engineered vessel grafts, Adv. Healthcare Mater. (2024) e2304432.
[11] Y. Wei, H. Jiang, C. Chai, P. Liu, M. Qian, N. Sun, et al., Endothelium-mimetic surface modification improves antithrombogenicity and enhances patency of vascular grafts in rats and pigs, JACC Basic Transl Sci 8 (7) (2023) 843–861.
[12] J.A. Mitchell, F. Ali, L. Bailey, L. Moreno, L.S. Harrington, Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium, Exp. Physiol. 93 (1) (2008) 141–147.
[13] P. Li, W. Cai, K. Wang, L. Zhou, S. Tang, Y. Zhao, et al., Selenium-functionalized polycarbonate-polyurethane for sustained in situ generation of therapeutic gas for blood-contacting materials, Smart Materials in Medicine 3 (2022) 361–373.
[14] X. Yang, B. Geng, J. Yan, L. Lin, X. Zhao, H. Xiao, et al., The role of exosomes in regulation and application of vascular homeostasis and vascular grafts, Smart Mater. Med 4 (2023) 538–551.
[15] J. Amersfoort, G. Eelen, P. Carmeliet, Immunomodulation by endothelial cells — partnering up with the immune system? Nat. Rev. Immunol. 22 (9) (2022) 576–588.
[16] Y. Yao, A.M. Zaw, D.E.J. Anderson, M.T. Hinds, E.K.F. Yim, Fucoidan functionalization on poly(vinyl alcohol) hydrogels for improved endothelialization and hemocompatibility, Biomaterials 249 (2020) 120011.
[17] Y. Wei, Y. Wu, R. Zhao, K. Zhang, A.C. Midgley, D. Kong, et al., Msc-derived sevs enhance patency and inhibit calcification of synthetic vascular grafts by immunomodulation in a rat model of hyperlipidemia, Biomaterials 204 (2019) 13–24.
[18] Y. Zhuang, C. Zhang, M. Cheng, J. Huang, Q. Liu, G. Yuan, et al., Challenges and strategies for in situ endothelialization and long-term lumen patency of vascular grafts, Bioact. Mater. 6 (6) (2021) 1791–1809.
[19] E. Ben-Akiva, R.A. Meyer, H. Yu, J.T. Smith, D.M. Pardoll, J.J. Green, Biomimetic anisotropic polymeric nanoparticles coated with red blood cell membranes for enhanced circulation and toxin removal, Sci. Adv. 6 (16) (2020) eaay9035.
[20] W. Chen, Q. Zhang, B.T. Luk, R.H. Fang, Y. Liu, W. Gao, et al., Coating nanofiber scaffolds with beta cell membrane to promote cell proliferation and function, Nanoscale 8 (19) (2016) 10364–10370.
[21] N. Pallet, I. Sirois, C. Bell, L.A. Hanafi, K. Hamelin, M. Dieudé, et al., A comprehensive characterization of membrane vesicles released by autophagic human endothelial cells, Proteomics 13 (7) (2013) 1108–1120.
[22] J. Shi, X.Y. Zhang, L. Jiang, L. Zhang, Y.S. Dong, A.C. Midgley, et al., Regulation of the inflammatory response by vascular grafts modified with aspirin triggered resolvin d1 promotes blood vessel regeneration, Acta Biomater. 97 (2019) 360–373.
[23] N.R. Mohamad, N.H. Marzuki, N.A. Buang, F. Huyop, R.A. Wahab, An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes, Biotechnol. Biotechnol. Equip. 29 (2) (2015) 205–220.
[24] Q. Wu, W. Wang, C. Zhang, Z. You, Y. Zeng, Y. Lu, et al., Capturing nascent extracellular vesicles by metabolic glycan labeling-assisted microfluidics, Nat. Commun. 14 (1) (2023) 6541.
[25] J. Han, R. Bhatta, Y. Liu, Y. Bo, A. Elosegui-Artola, H. Wang, Metabolic glycan labeling immobilizes dendritic cell membrane and enhances antitumor efficacy of dendritic cell vaccine, Nat. Commun. 14 (1) (2023) 5049.
[26] F. Tang, M. Zhou, K. Qin, W. Shi, A. Yashinov, Y. Yang, et al., Selective n-glycan editing on living cell surfaces to probe glycoconjugate function, Nat. Chem. Biol. 16 (7) (2020) 766–775.
[27] L. Nie, Y. Sun, O.V. Okoro, Y. Deng, G. Jiang, A. Shavandi, Click chemistry for 3d bioprinting, Mater. Horiz. 10 (8) (2023) 2727–2763.
[28] R. Bhatta, J. Han, Y. Liu, Y. Bo, D. Lee, J. Zhou, et al., Metabolic tagging of extracellular vesicles and development of enhanced extracellular vesicle based cancer vaccines, Nat. Commun. 14 (1) (2023) 8047.
[29] W. Xiao, W. Chen, Y. Wang, C. Zhang, X. Zhang, S. Zhang, et al., Recombinant dtβ4-inspired porous 3d vascular graft enhanced antithrombogenicity and recruited circulating cd93(+)/cd34(+) cells for endothelialization, Sci. Adv. 8 (28) (2022) eabn1958.
[30] D. Hao, Y. Fan, W. Xiao, R. Liu, C. Pivetti, T. Walimbe, et al., Rapid endothelialization of small diameter vascular grafts by a bioactive integrin-binding ligand specifically targeting endothelial progenitor cells and endothelial cells, Acta Biomater. 108 (2020) 178–193.
[31] Y. Yang, P. Gao, J. Wang, Q. Tu, L. Bai, K. Xiong, et al., Endothelium-mimicking multifunctional coating modified cardiovascular stents via a stepwise metal-catechol-(amine) surface engineering strategy, Research (2020) 9203906.
[32] W.-J. Ho, M. Kobayashi, K. Murata, Y. Hashimoto, K. Izumi, T. Kimura, et al., A novel approach for the endothelialization of xenogeneic decellularized vascular tissues by human cells utilizing surface modification and dynamic culture, Sci. Rep. 12 (1) (2022) 22294.
[33] Q. Cai, W. Liao, F. Xue, X. Wang, W. Zhou, Y. Li, et al., Selection of different endothelialization modes and different seed cells for tissue-engineered vascular graft, Bioact. Mater. 6 (8) (2021) 2557–2568.
[34] D. Zhi, Q. Cheng, A.C. Midgley, Q. Zhang, T. Wei, Y. Li, et al., Mechanically reinforced biotubes for arterial replacement and arteriovenous grafting inspired by architectural engineering, Sci. Adv. 8 (11) (2022) eabl3888.
[35] H. Wang, M. Xing, W. Deng, M. Qian, F. Wang, K. Wang, et al., Anti-sca-1 antibody-functionalized vascular grafts improve vascular regeneration via selective capture of endogenous vascular stem/progenitor cells, Bioact. Mater. 16 (2022) 433–450.
[36] M. Rafique, T. Wei, Q. Sun, A.C. Midgley, Z. Huang, T. Wang, et al., The effect of hypoxia-mimicking responses on improving the regeneration of artificial vascular grafts, Biomaterials 271 (2021) 120746.
[37] D. Pretorius, R.P. Richter, T. Anand, J.C. Cardenas, J.R. Richter, Alterations in heparan sulfate proteoglycan synthesis and sulfation and the impact on vascular endothelial function, Matrix Biol. 16 (2022) 100121.
[38] L.E. Collins, L. Troeberg, Heparan sulfate as a regulator of inflammation and immunity, J. Leukoc. Biol. 105 (1) (2019) 81–92.
[39] V. Chugh, K. Vijaya Krishna, A. Pandit, Cell membrane-coated mimics: a methodological approach for fabrication, characterization for therapeutic applications, and challenges for clinical translation, ACS Nano 15 (11) (2021) 17080–17123.
[40] Y. Takayama, K. Kusamori, M. Nishikawa, Click chemistry as a tool for cell engineering and drug delivery, Molecules 24 (1) (2019) 172.
[41] E. Mueller, I. Poulin, W.J. Bodnaryk, T. Hoare, Click chemistry hydrogels for extrusion bioprinting: progress, challenges, and opportunities, Biomacromolecules 23 (3) (2022) 619–640.
[42] L.E. Niklason, J.H. Lawson, Bioengineered human blood vessels, Science 370 (6513) (2020) eaaw8682.
[43] B.B.J. Leal, N. Wakabayashi, K. Oyama, H. Kamiya, D.I. Braghirolli, P. Pranke, Vascular tissue engineering: polymers and methodologies for small caliber vascular grafts, Front Cardiovasc Med 7 (2020) 592361.