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REVIEW

Endothelial colony-forming cells in the spotlight: insights into the pathophysiology of von Willebrand disease and rare bleeding disorders

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Abstract

Endothelial cells deliver a vital contribution to the maintenance of hemostasis by constituting an anatomical as well as functional barrier between the blood and the rest of the body. Apart from the physical barrier function, endothelial cells maintain the hemostatic equilibrium by their pro- and anticoagulant functions. An important part of their procoagulant contribution is the production of von Willebrand factor (VWF), which is a carrier protein for coagulation factor VIII and facilitates the formation of a platelet plug. Thus, VWF is indispensable for both primary and secondary hemostasis, which is exemplified by the bleeding disorder von Willebrand disease that results from qualitative or quantitative deficiencies in VWF. A cellular model that was found to accurately reflect the endothelium and its secretory organelles are endothelial colony-forming cells, which can be readily isolated from peripheral blood and constitute a robust *ex vivo* model to investigate the donor's endothelial cell function. This review summarizes some of the valuable insights on biology of VWF and pathogenic mechanisms of von Willebrand disease that have been made possible using studies with endothelial colony-forming cells derived from patients with bleeding disorders.

KEYWORDS
blood coagulation disorder, endothelial cells, von Willebrand diseases, von Willebrand factor, Weibel–Palade bodies

1 | INTRODUCTION

Hemostasis is the cessation of bleeding through coagulation in the event of vascular injury. The hemostatic balance is an interplay of procoagulant and anticoagulant mechanisms and can be regarded as a balance. Tipping too far to one side without sufficient adjustments in response ultimately leads to defective or overactivated coagulation, which increases the risk of bleeding or thrombosis, respectively. Much of the research in this field has been and still is revolving around identifying and investigating (new) hemostatic players with the aim of unraveling their underlying mechanisms and how their interplay can give rise to a pathogenic state. In this review, we will briefly outline how endothelial

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cells (ECs) actively participate in preserving the hemostatic balance, with particular focus on their role in regulating acute and steady-state levels of the hemostatic protein von Willebrand factor (VWF). Our molecular understanding of the biosynthesis and secretion of VWF primarily originates from *in vitro* studies in ECs. Here, we will highlight endothelial colony-forming cells (ECFCs) as a versatile, *ex vivo* EC model for studying basic principles of EC biology and hemostasis in their native environment. ECFCs are uniquely suited to study the links between genetic mutations in patients and their cellular phenotype. This will be illustrated by the insights obtained from patient-derived ECFCs, such as for the bleeding disorder von Willebrand disease (VWD), the process of angiogenesis, and for regulated secretion of VWF via Weibel–Palade body (WPB) exocytosis.

2 | THE ROLE OF ECS IN HEMOSTASIS

ECs passively and actively contribute to anti- and procoagulant as well as fibrinolytic mechanisms (Figure 1). Their anticoagulant function is vital as it prevents blood from unintentional clotting and, in doing so, averts the formation of clots that can cause thrombosis, infarcts, or stroke. The endothelium acts first and foremost as a physical barrier between blood and tissue. As such, it also prevents contact of platelets and coagulation factors in the circulation with procoagulant components in the subendothelial matrix, such as collagen and tissue factor (TF), thereby restricting initiation of primary and secondary hemostasis pathways, respectively [1].

ECs also take more active roles in preserving the hemostatic balance. Nitric oxide and prostacyclin (prostaglandin I2), which are continuously synthesized by ECs, are potent vasodilators and platelet inhibitors that are used for local control of hemodynamic forces in the blood and adhesion and aggregation of platelets [2,3]. ECs also produce TF pathway inhibitor, which blocks the TF-activated factor (F)VII complex and prothrombinase of the extrinsic pathway [4]. Additionally, ECs can act on the intrinsic pathway by producing and presenting anticoagulants like heparan sulfate proteoglycans on their membranes, which bind liver-produced antithrombin to the vessel wall. This induces conformational changes in antithrombin and enhances its interaction with the blood

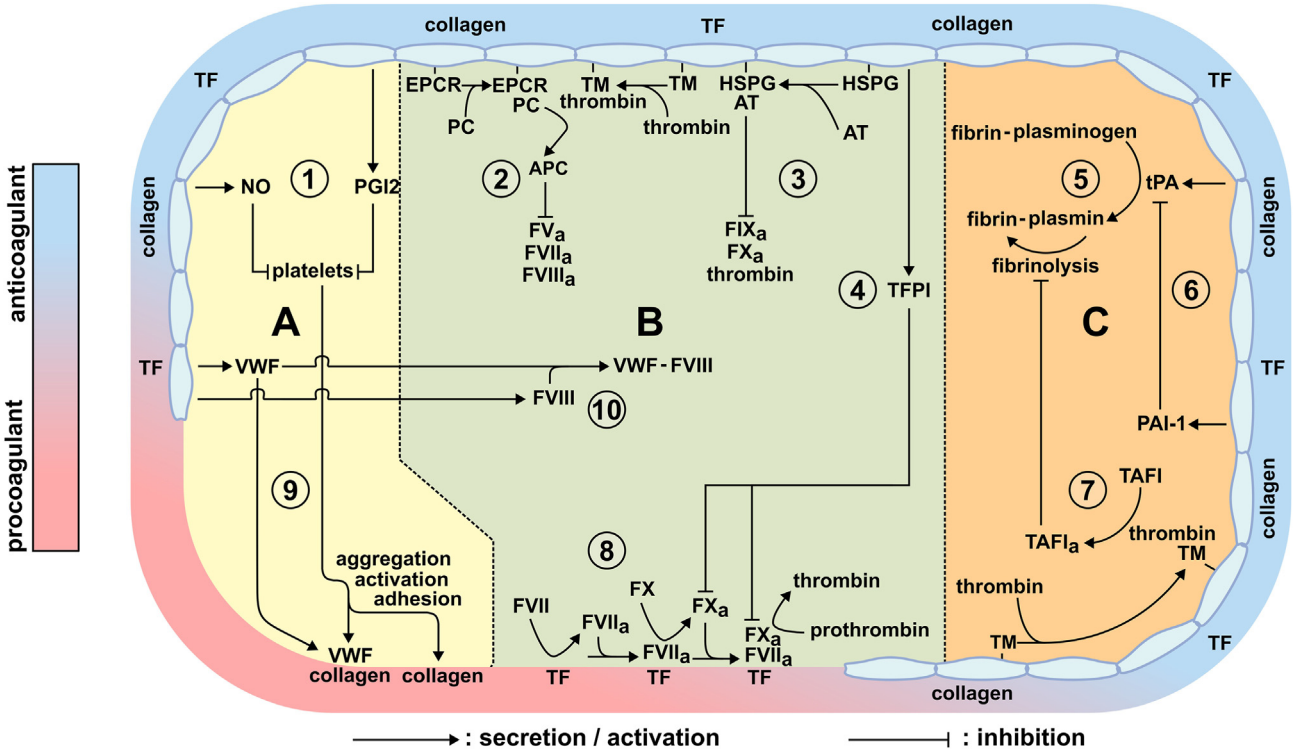

FIGURE 1 Anticoagulant and procoagulant functions of endothelial cells. Endothelial cells (ECs) directly and indirectly contribute to anticoagulant and procoagulant mechanisms in (A) primary and (B) secondary hemostasis and (C) fibrinolysis. Anticoagulant mechanisms in primary and secondary hemostasis include synthesis of the platelet inhibitors nitric oxide (NO) and prostaglandin I2 (PGI2; 1), the protein C (PC) pathway supported by the endothelial PC receptor (EPCR)–thrombomodulin (TM) tandem on ECs (2), the antithrombin (AT) pathway on heparan sulfate proteoglycans (HSPGs) on ECs (3), and the synthesis of tissue factor pathway inhibitor (TFPI), which negatively regulates the tissue factor (TF) pathway (4). EC control over fibrinolysis includes secretion of tissue plasminogen activator (tPA), which activates plasminogen on fibrin into plasmin, thereby promoting the degradation of fibrin (5). Activation of fibrinolysis is counteracted by plasminogen activator inhibitor-1 (PAI-1) produced by ECs (6) and the conversion of thrombin-activatable fibrinolysis inhibitor (TAFI) by the thrombin-TM complex on ECs (7). Damage to the vessel wall exposes TF within the subendothelial matrix and activates the TF pathway, resulting in the generation of thrombin (8). Binding of von Willebrand factor (VWF) to exposed collagen promotes adhesion and subsequent activation and aggregation of platelets (9), while binding of VWF to factor (F)VIII prevents FVIII from premature clearance (10). APC, activated protein C.

clotting cascade proteinases FXa and FIXa to inhibit their activity [5]. Additionally, ECs modulate the common pathway through their production of thrombomodulin, which is a thrombin-specific receptor on the EC membrane that decreases circulating thrombin levels. Moreover, ECs express EC protein C (PC) receptors on their membranes, which can bind to PC. This facilitates activation of PC into activated PC by the thrombin-thrombomodulin complex, which leads to the subsequent inactivation of coagulation factors such as FV, FVII, and FVIII [6].

Stimulated and constitutive secretion of tissue plasminogen activator (tPA) from ECs [7,8] leads to the conversion of fibrin-bound plasminogen to plasmin, which is a serine protease that degrades fibrin during fibrinolysis and clot resolution [9]. ECs also express inhibitors of fibrinolysis, such as serpin plasminogen activator inhibitor-1 [10], which inhibits tPA by binding its active site, thereby preventing the interaction of tPA with plasminogen and the latter's subsequent conversion to fibrin [11]. Furthermore, the thrombin-thrombomodulin complex, which is found on the surface of ECs, activates the thrombin-activatable fibrinolysis inhibitor. Thrombin-activatable fibrinolysis inhibitor cleaves C-terminal lysine residues from fibrin, which decreases its affinity for plasminogen and tPA, reduces plasmin generation, and thus attenuates fibrinolysis [12].

The procoagulant function of the endothelium is mainly mediated by VWF, which is secreted by ECs and plays a central role in primary and secondary hemostasis. At steady state, VWF is critical for the maintenance of sufficient levels of circulating FVIII by physically preventing the latter's premature clearance from plasma [13]. Following vascular injury and exposure of the subendothelial matrix, VWF circulating in plasma as well as VWF that is locally secreted by activated ECs, binds to collagen via its A3 domain. As a result of the hemodynamic forces of the blood flow, these tethered VWF strings unfold and expose a binding site for the platelet GP1bα receptor within the VWF A1 domain that supports the adhesion and subsequent activation and aggregation of platelets to sites of vascular injury, a critical step in the formation of a platelet plug [14]. The importance of VWF is highlighted by the mild to severe bleeding abnormalities that occur in patients with (partial) quantitative reduction or a qualitative defect of VWF circulating in plasma, as seen in VWD and in what was formerly known as "Low VWF" [15,16]. Partial deficiencies of functionally normal VWF are often the result of *VWF* missense mutations [17] that reduce its synthesis and/or secretion or lead to enhanced clearance from the circulation as evidenced by altered VWF propeptide (VWFpp)/VWF antigen and FVIII activity/VWF antigen ratios in plasma [18]. However, approximately 30% to 50% of patients with quantitative VWF reductions lack pathogenic *VWF* variants [18–21]. In these cases, other genetic modifiers, including those acting on clearance, synthesis, and secretion, may be responsible for the reduced VWF levels [22].

3 | CELL BIOLOGY OF VWF

ECs are responsible for the production of nearly all VWF that is found in plasma [23]. VWF is first synthesized as a pre-proVWF monomer, consisting of an N-terminal signal peptide, the VWFpp, and the mature VWF subunit, which contains its ligand-binding domains and domains responsible for dimerization and multimerization [24]. Following signal peptide cleavage and dimerization of proVWF monomers in the endoplasmic reticulum (ER), the acidic conditions in the trans-Golgi network promote concatemerization of VWF dimers along helical self-templates, leading to the formation of long tubules that consist of ultralarge VWF multimers [25–27]. Expression of VWF drives the formation of WPBs, EC-specific secretory organelles that are formed at the trans-Golgi network and store VWF for basal or stimulated release [28–30]. WPBs are generally 1 to 5 μm long, 0.1 to 0.3 μm wide, and owe their characteristic rod shape to the dimensions and parallel arrangement of the VWF tubules [26,30,31].

Newly synthesized WPBs are considered immature and lack the ability to undergo stimulus-induced secretion [32]. WPBs go through a post-Golgi maturation process that involves the recruitment of proteins to the WPB membrane and a further acidification to pH ~5.4 [30,33]. A key step in this process is the acquisition of Rab GTPases Rab27A and isoforms of Rab3 [32,34,35], which depends on their activation by the guanine nucleotide exchange factor MAP kinase activating death domain [36]. In turn, active Rabs on the membrane of mature WPBs recruit effectors that interact with soluble N-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) proteins and other exocytotic components that perform essential functions in cytoskeletal interactions, organelle acidification, and exocytosis of WPBs [32,37–46]. As a result of recruitment of their secretory machinery, WPBs become secretion-competent and can undergo exocytosis upon activation of the EC, such as during vascular injury [30]. Apart from VWF, WPBs are known to store a variety of other vasoactive agents, including chemokines, angiogenic mediators such as insulin-like growth factor binding protein 7 (IGFBP7) and angiopoietin-2 (Ang-2), as well as hemostatic proteins FVIII and tPA (reviewed in the study by Hordijk et al. [30]). Corelease of these proteins together with VWF may help the endothelium to further fine-tune its hemostatic responses while simultaneously initiating inflammatory and blood vessel repair pathways.

The SNARE proteins syntaxin-2 and syntaxin-binding protein 5, which are part of this machinery, have previously been identified in quantitative trait locus mapping studies for genetic associations with VWF [47–50], suggesting that alterations in the exocytotic process of WPBs contribute to the wide range of plasma VWF levels in the population. Together, this highlights the potential role of the endothelial secretory pathway as a major determinant of circulating VWF levels and bleeding phenotype.

4 | CELLULAR MODEL SYSTEMS

With the growing insight from large-scale genomic screenings into the genetic mechanisms that underpin VWF abnormalities, investigators now face the challenge to find experimental evidence of the functional impact of variants in *VWF* or other genetic determinants that explains alterations in VWF found in patients. Over the years, a number of

approaches have been developed to test these genotype-phenotype relationships in model systems that, to a varying extent, reflect the cellular context in which these pathogenic mechanisms play out. Expression of VWF in non-EC types such as Chinese hamster ovary and human embryonic kidney 293 cell lines has long been the method of choice to study how pathogenic VWF variants affect its biosynthesis, posttranslational modification, and molecular composition. Because ectopic expression of VWF induces the formation of so-called pseudo-WPBs [51,52], elongated storage organelles that have a striking resemblance to WPBs in terms of morphology and composition, these studies can also reveal how mutations translate to altered storage and secretion of VWF and other WPB constituents [52–54]. Such studies do require *a priori* knowledge of the mutations involved and can be difficult to interpret, considering that the vast majority (up to 90%) of pathogenic *VWF* variants in VWD are heterozygous dominant-negative mutations [17] that express in conjunction with a normal functional allele in patients. Moreover, other caveats of studying VWF biology outside of its proper cellular context include lack of EC-specific gene expression, nonphysiological expression levels from expression constructs with constitutive promoters, as well as the absence of a regulated secretory pathway and many components of the associated exocytotic machinery. This makes ectopic systems such as human embryonic kidney 293 cells of very limited value for functional studies of stimulated VWF secretion or angiogenesis.

To study these processes in their native cellular context, it is inevitable to turn to ECs, ideally from the patients themselves. Primary ECs from a multitude of vascular beds, such as from the dermis, brain, lungs, heart, aorta, retina, umbilical cords, and foreskin, are nowadays commercially available. Nonetheless, the invasive procedures involved in isolating these endothelial subtypes from patients would make most of them impractical for study purposes. Differentiation of ECs from patient-derived induced pluripotent stem cells could potentially overcome such hurdles, but varying levels of VWF synthesis between clonal lines and impaired maturation of WPBs [55] are currently still precluding ECs from patient-derived induced pluripotent stem cells from becoming a viable model system for this purpose.

ECs can also be derived from a population of cells that circulates in blood, so-called endothelial progenitor cells, which can be divided into 2 populations, early and late, based on their appearance in culture. Late-appearing endothelial progenitor cells, first described by Lin et al. [56], are of *bona fide* endothelial lineage as judged by the expression of a set of canonical endothelial markers, including VWF, vascular endothelial growth factor (VEGF) receptor-2, PECAM, and VE-cadherin, and several EC-specific properties such as angiogenic capacity. These cells have been referred to by various other names, including endothelial outgrowth cells and blood outgrowth ECs. To bring order to the nomenclature conundrum, a consensus was reached to rename them ECFCs [57], which is also the terminology we will use in this review.

ECFCs can be regarded as liquid biopsies of the endothelium [58] and are excellent model systems to investigate pathogenic cellular mechanisms that affect the vasculature since they carry the genetic background of the patient [59]. Despite the fact that their exact origin is still a matter of debate [60,61], ECFCs can be acquired quite reliably from various sources, including venous blood [56,62,63], cord blood [64,65], and even cryopreserved peripheral blood mononuclear cells [66]. However, some concerns exist regarding their intraindividual and interindividual phenotypic heterogeneity [67,68], which can potentially influence outcomes of studies and may have its basis in a progression of some ECFC lines into an endothelial-to-mesenchymal transition state [69].

Despite these challenges, ECFCs are a robust model to directly assess how disease genotype translates to endothelial phenotype *ex vivo*, which is invaluable for research on pathologies that are characterized by an affected endothelium, such as VWD.

5 | ECFCS: VWD

The first use of VWD patient–derived ECFCs was by Berber et al. [70], who investigated the common R924Q polymorphism in ECFCs of a compound heterozygous VWD type 2N patient (R816W/R924Q) but failed to find conclusive evidence for the pathogenic nature of this variant. Back-to-back publications by Starke et al. [71] and Wang et al. [72] followed shortly after, who used ECFCs from type 1 and type 2 VWD patients to investigate the cellular phenotypes that are associated with a variety of exonic mutations within *VWF*. This marked a key advance in the field as the consequences of the pathogenic mechanisms involved in VWD could be revealed to their full extent for the first time. Depending on the mutations involved, these mechanisms include retention of VWF inside the ER, incorrect proteolytic processing, reduced high-molecular-weight VWF multimers, impaired stimulus-induced secretion, the loss of the characteristic elongated morphology of WPBs, and reduction or loss of these organelles entirely. Moreover, Wang et al. [72] showed that in selected cases, these defects can lead to reduced ability to generate long VWF strings upon release from the endothelium, which is expected to exacerbate bleeding complications in patients who already have reduced circulating VWF levels. Later studies focusing on ECFCs from VWD type 3 patients, as well as patients with mutations in the VWFpp region, the C-terminal cysteine knot domain, or with large in-frame deletions, also reported retention of VWF in the ER in conjunction with reduced numbers of small, spherical WPBs [73–76], underlining that this is a common pathogenic mechanism in quantitative and qualitative VWD (Figure 2). Since unrestrained progression of VWF through the secretory pathway is important for the generation of sufficient numbers of elongated WPBs that are capable of secreting long VWF strings [77], any variant of VWF that causes (partial) retention in the ER can be expected to be accompanied by impaired secretory responses.

The VWD mutational spectrum also includes a large number of noncoding and splice site mutations for which the pathogenic mechanisms long remained elusive. An early study using VWD ECFCs detailed the mechanism by which a heterozygous 13 bp deletion in the *VWF* promoter in a VWD type 1 patient brought on reduced VWF transcript production from that allele, thereby showing for the first time how VWF promoter mutations can lead to quantitative VWF

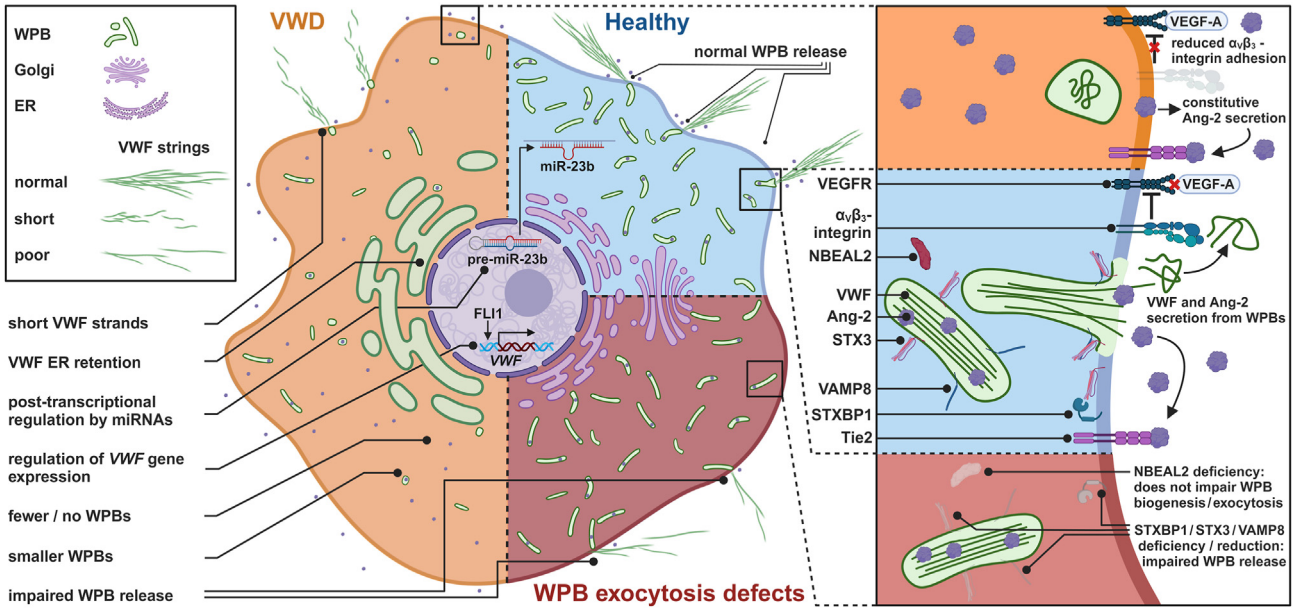

FIGURE 2 Insights into the pathophysiology of bleeding disorders from studying endothelial colony-forming cells. The model depicts 3 states of an endothelial cell: in healthy condition (blue), when derived from a patient with von Willebrand disease (VWD; orange), or in other diseases where Weibel–Palade body (WPB) secretion defects occur (red). On the right, a zoomed-in panel displays components of the secretory pathway and how it may be affected in patients with bleeding disorders. $\alpha_V\beta_3$, $\alpha_V\beta_3$-integrin; Ang-2, angiopoietin-2; ER, endoplasmic reticulum; FLI1, friend leukemia integration 1; miRNA, microRNA; miR-23b, microRNA 23b; NBEAL2, neurobeachin-like 2; STX3, syntaxin 3; STXBP1, syntaxin-binding protein 1; VAMP8, vesicle-associated membrane protein 8; VEGF-A, vascular endothelial growth factor A; VEGFR, vascular endothelial growth factor receptor; VWF, von Willebrand factor. Created with BioRender.com.

deficiencies [78]. Additional studies using VWD patient ECFCs unraveled the pathogenic mechanisms of splice site mutations, a silent exonic mutation that led to intron retention, and a deep intronic mutation [79–81]. It is worth noting that some of these studies involving *VWF* mutations in noncoding regions or splice site mutations would have been impossible in ectopic expression systems.

VWD ECFCs have also been used to identify new genetic modifiers of *VWF* that could contribute to quantitative deficiencies of VWF. Two studies used transcriptomic analysis of ECFCs from patients with "Low VWF" and VWD type 1 [82,83], identifying the transcription factor friend leukemia integration 1 and the microRNA miR-23b as potential modifiers of *VWF* transcription and stimulated VWF secretion, respectively. Such screenings are of a hypothesis-generating nature and will require further experimental validation of candidates to assess their biological and clinical relevance.

Finally, patient-derived ECFCs have significant potential as an *ex vivo* model for the development of new therapeutics for VWD, for instance, as a platform to test strategies for permanent or temporal correction of VWF defects. Proof of principle for phenotypic correction was shown in VWD type 3 dog ECFCs that were transduced with lentivirus carrying VWF complementary DNA [84]. More recently, small interfering RNAs targeting exonic single nucleotide polymorphisms in *VWF* were used to allele-selectively silence a heterozygous p.C1190Y mutation in ECFCs of a VWD type 2A patient, resulting in loss of mutant allele expression and phenotypic correction *ex vivo* [85]. This could pave the way for more individualized approaches by using ECFCs as an *ex vivo* validation model for possible VWD treatments.

6 | ECFCS: VWD AND ANGIOGENESIS

Angiodysplastic lesions in the gastrointestinal (GI) tract are fragile vascular networks that are associated with recurrent GI bleeding, which can result in anemia and a decrease in quality of life [86]. In up to 38% of VWD patients with GI bleeding, angiodysplastic lesions are present in the GI tract [87], which pose significant challenges in combination with the bleeding disorder [88]. Angiodysplasia is the result of abnormal angiogenesis, the process that generates new blood vessels from existing vasculature. Directed migration and reorganization of ECs during angiogenesis is highly dependent on growth factors such as VEGF, angiopoietin-1, and Ang-2. VEGF is a potent stimulus of VWF secretion from WPBs [89], which also store the Tie2 receptor ligand Ang-2 and other proangiogenic components such as IGFBP7 [90,91]. Targeted release of Ang-2 by exocytosis of WPBs activates autocrine and paracrine Tie2 signaling, which contributes to vessel lumen formation and shaping of new blood vessels [44,92,93]. VWF is also a ligand for adhesion receptors such as $\alpha_V\beta_3$-integrin, which is an important regulator of EC migration that can also quench VEGF receptor-2 signaling when in complex with ligands such as VWF [94]. VWF binds a large array of proangiogenic growth factors via its heparin-binding domain [95] and also binds Ang-2 and IGFBP7 after release [91,96,97], which may serve as a mechanism to focus these factors in areas of wound healing and active blood vessel formation.

The first evidence for a role in angiogenesis for VWF was presented by Starke et al. [98], who found increased migration,

proliferation, and angiogenesis in ECs that were depleted of VWF using RNA interference and in ECFCs from a cohort of VWD type 1, 2M, and 2A patients. This was accompanied by reduced β_3-integrin expression, reduced $\alpha_V\beta_3$-integrin–dependent adhesion, and increased constitutive Ang-2 secretion, possibly as a result of reduced intracellular retention of Ang-2 due to loss of WPBs. A second study by Groeneveld et al. [99] reported that compared with control ECFCs, most type 1 and type 2 VWD ECFCs had a slightly lower directionality of migration in a wound-healing assay, while VWD type 3 ECFCs, which are entirely devoid of VWF synthesis, had a higher migration velocity. However, no clear effects of *VWF* mutations on overall angiogenic potential were seen in this VWD cohort. Selvam et al. [100] found wide variation in parameters such as migration, proliferation, and tube formation in VWD patient–derived ECFCs but also reported that type 1, type 2, and some type 3 VWD ECFCs had significantly increased constitutive Ang-2 secretion compared with controls. A similar observation was made in cord blood ECFCs in which clustered regulalry interspaced short palindromic repeats/Cas9-mediated *VWF* gene knockout led to increased constitutive secretion of Ang-2 and association of Ang-2 with the Tie2 receptor [101]. Since these cells are also devoid of WPBs and no longer capable of storing Ang-2, stimulus-induced Ang-2 release was severely reduced. All together, these (patient-) ECFC-based studies have provided some evidence for a role of VWF in angiogenesis (Figure 2). However, the exact function remains unclear, perhaps due to the limitations of this model, which are associated with the phenotypic heterogeneity and gradual loss of proliferative capacity of patient and control ECFCs.

7 | ECFCS: WPB EXOCYTOSIS DEFECTS AND STORAGE POOL DISORDERS

The cellular machinery involved in biogenesis and secretion of WPBs is complex [30]. Because regulated secretion is fundamental to the function of numerous different cell types, many components of the WPB machinery are also involved in similar secretory processes in non-ECs. Defects in shared secretory components can therefore affect more than 1 cell type and may lead to complex, multisystem manifestations in patients. In several cases, ECs isolated from rare patients with abnormalities in non-EC functions have contributed to identification and functional characterization of new regulators of WPB secretion (Figure 2). The SNARE proteins syntaxin-2, syntaxin-3 (STX3), and syntaxin-binding protein 1 (STXBP1) were identified as hits in an interactomic screen of downstream effectors of the Rab27A-Slp4-a complex in ECs. *De novo* mutations in *STXBP1* are associated with early infantile epileptic encephalopathy type 4, an epileptic disorder that is thought to result from impaired neurotransmitter release due to STXBP1 haploinsufficiency. In keeping with the defective secretory responses in neurons in these patients, STXBP1 haploinsufficient epileptic encephalopathy type 4 patient–derived ECFCs also showed severely impaired Ca^{2+}- and cAMP-mediated VWF secretion, confirming the role of STXBP1 in stimulus-induced WPB exocytosis [39]. Homozygous nonsense mutations in *STX3* are causative for microvillus inclusion disease, a rare congenital disorder of the gut characterized by severe diarrhea, which is the result of incorrect targeting of microvilli that normally migrate/move to the apical side of intestinal epithelial cells [102]. Microvillus inclusion disease patient–derived ECFCs, which were entirely devoid of STX3, had reduced VWF and VWFpp secretion at submaximal stimulation and strikingly showed signs of loss of polarity of VWF and VWFpp release during basal secretion [42].

Storage pool disorders (SPDs) are a heterogeneous group of disorders that affect the formation of lysosome-related organelles (LROs), which include not only platelet α-granules and dense granules but also WPBs [103]. Due to the universal mechanisms involved in the formation of LROs, SPDs often present as multisystem disorders and affect secretory function of both platelets and a variety of other cell types. Hermansky–Pudlak syndrome 2 (HPS2) is a rare genetic SPD characterized by interstitial lung disease, neutropenia, and bleeding, which may find its origin in the lack of dense granules in platelets. HPS2 is caused by mutations in *AP3B1*, which encodes for the β subunit of the adaptor-related protein 3 (AP-3) complex, a cargo-sorting complex that traffics secretory cargo and membrane proteins from endosomes to LROs. Whole proteome analysis of ECFCs derived from HPS2 patients revealed that loss of the AP-3 complex due to compound heterozygous *AP3B1* mutations was accompanied by loss of vesicle-associated membrane protein 8 (VAMP8) [104]. VAMP8 is an R-SNARE that cycles via an endosomal compartment to WPBs, where it supports the exocytotic fusion of WPBs via interaction with plasma membrane-based Q-SNAREs. Lacking VAMP8 on their WPBs, HPS2 ECFCs showed severely reduced stimulus-induced WPB exocytosis, suggesting that WPBs acquire secretion competence partly by recruitment of membrane proteins such as SNAREs from the endosomal compartment in an AP-3–dependent manner. It also indicates that the bleeding abnormalities that are seen in some SPDs and that were generally attributed to impaired platelet function may in some cases be further compounded by decreased endothelial secretory responses. This can however not be extrapolated to all SPDs. Kat et al. [105] found that NBEAL2-deficient ECFCs from patients with Gray platelet syndrome, a bleeding disorder characterized by loss of platelet α-granules as well as abnormalities in formation of secretory organelles in neutrophils, had normal biogenesis, maturation, and exocytosis of WPBs. This also underscores the divergent mechanisms that control VWF storage in endothelial WPBs and in α-granules in megakaryocytes. In aforementioned studies, the ECFCs originate from individuals suffering from rare genetic disorders. While these results come from very small sample sizes, they can still be very useful to unravel complex cellular mechanisms of disease.

8 | THE ROAD AHEAD FOR ECFCS

ECFCs are unique personalized endothelial model systems to study EC function in health and disease and have already led to major advances in our understanding of VWD and WPB biology (Figure 2). While they have generated great interest in the hemostasis community and various other focus areas of vascular biology, their use is still

complicated by poor standardization of isolation and culturing methods and substantial intraindividual and interindividual phenotypic variability. This can negatively impact the interpretability of experimental results, which for now may preclude their wider adaptation within the scientific community. Future efforts should be aimed at understanding the source of this variation and designing strategies that can prevent or minimize its impact on experimental outcome. Disease modeling using ECFCs, such as described here for VWD, has so far been limited to studying these cells in isolation using 2-dimensional cell culture platforms. To fully appreciate EC function within its authentic (patho-)physiological context, for instance, within an injured blood vessel or during organotypic function, it is pertinent that the physical and chemical milieu that is induced by blood flow or neighboring cells is present. Integration of ECFCs with vessel- or organ-on-a-chip technologies offers the exciting opportunity to develop miniature *ex vivo* personalized disease models that include the patient's own diseased endothelium.

APPENDICES

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The SYMPHONY consortium, which aims to orchestrate personalized treatment in patients with bleeding disorders, is a unique collaboration between patients, health care professionals, and translational and fundamental researchers specializing in inherited bleeding disorders, as well as experts from multiple disciplines [106]. It aims to identify the best treatment choice for each individual based on bleeding phenotype. To achieve this goal, work packages (WP) have been organized according to 3 themes (eg, Diagnostics [WPs 3 and 4], Treatment [WPs 5-9], and Fundamental Research [WPs 10-12]). Principal investigator: Marjon H. Cnossen; project manager: Simone H. Reitsma. Funding by SYMPHONY: Dutch Research Agenda - Dutch Research Council (NWO-NWA) 1160.18.038 (received by S.N.J.L., R.B., and J.C.J.E.) and Landsteiner Foundation for Blood Transfusion Research, LSBR-1707 and -2005 (received by R.B.).

AUTHOR CONTRIBUTIONS

S.N.J.L., B.G.L., and R.B. set up the literature review and wrote the paper. S.N.J.L., B.G.L., J.C.J.E., and R.B. critically revised the intellectual content. All authors approved the final manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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REFERENCES

[1] Versteeg HH, Heemskerk JWM, Levi M, Reitsma PH. New fundamentals in hemostasis. *Physiol Rev*. 2013;93:327–58.
[2] Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet*. 1987;2:1057–8.
[3] Moncada S, Higgs EA, Vane JR. Human arterial and venous tissues generate prostacyclin (prostaglandin x), a potent inhibitor of platelet aggregation. *Lancet*. 1977;1:18–20.
[4] Mast AE. Tissue factor pathway inhibitor: multiple anticoagulant activities for a single protein. *Arterioscler Thromb Vasc Biol*. 2016;36:9–14.
[5] Olson ST, Richard B, Izaguirre G, Schedin-Weiss S, Gettins PGW. Molecular mechanisms of antithrombin-heparin regulation of blood clotting proteinases. A paradigm for understanding proteinase regulation by serpin family protein proteinase inhibitors. *Biochimie*. 2010;92:1587–96.
[6] Esmon CT. The protein C pathway. *Chest*. 2003;124(Suppl):26S–32S.
[7] Emeis JJ, Van Den Eijnden-Schrauwen Y, Van Den Hoogen CM, De Priester W, Westmuckett A, Lupu F. An endothelial storage granule for tissue-type plasminogen activator. *J Cell Biol*. 1997;139:245–56.
[8] Knipe L, Meli A, Hewlett L, Bierings R, Dempster J, Skehel P, Hannah MJ, Carter T. A revised model for the secretion of tPA and cytokines from cultured endothelial cells. *Blood*. 2010;116:2183–91.
[9] Longstaff C, Kolev K. Basic mechanisms and regulation of fibrinolysis. *J Thromb Haemost*. 2015;13(Suppl 1):S98–105.
[10] van Mourik JA, Lawrence DA, Loskutoff DJ. Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. *J Biol Chem*. 1984;259:14914–21.
[11] Morrow GB, Whyte CS, Mutch NJ. A serpin with a finger in many PAIs: PAI-1's central function in thromboinflammation and

cardiovascular disease. *Front Cardiovasc Med*. 2021;8:653655. https://doi.org/10.3389/fcvm.2021.653655

[12] Plug T, Meijers JC. Structure-function relationships in thrombin-activatable fibrinolysis inhibitor. *J Thromb Haemost*. 2016;14:633–44.

[13] Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. *Blood*. 2016;128:2007–16.

[14] Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. *J Thromb Haemost*. 2003;1:1335–42.

[15] Leebeek FWG, Eikenboom JCJ. Von Willebrand's disease. *N Engl J Med*. 2016;375:2067–80.

[16] Atiq F, Blok R, van Kwawegen CB, Doherty D, Lavin M, van der Bom JG, O'Connell NM, de Meris J, Ryan K, Schols SEM, Byrne M, Heubel-Moenen FCJI, van Galen KPM, Preston RJS, Cnossen MH, Fijnvandraat K, Baker RI, Meijer K, James P, Di Paola J, et al. Type 1 VWD classification revisited: novel insights from combined analysis of the LoVIC and WiN studies. *Blood*. 2024;143:1414–24.

[17] de Jong A, Eikenboom J. Von Willebrand disease mutation spectrum and associated mutation mechanisms. *Thromb Res*. 2017;159:65–75.

[18] Atiq F, Boender J, van Heerde WL, Tellez Garcia JM, Schoormans SC, Krouwel S, Cnossen MH, Laros-van Gorkom BAP, de Meris J, Fijnvandraat K, van der Bom JG, Meijer K, van Galen KPM, Eikenboom J, Leebeek FWG. Importance of genotyping in von Willebrand disease to elucidate pathogenic mechanisms and variability in phenotype. *Hemasphere*. 2022;6:e718. https://doi.org/10.1097/HS9.0000000000000718

[19] James PD, Notley C, Hegadorn C, Leggo J, Tuttle A, Tinlin S, Brown C, Andrews C, Labelle A, Chirinian Y, O'Brien L, Othman M, Rivard G, Rapson D, Hough C, Lillicrap D. The mutational spectrum of type 1 von Willebrand disease: results from a Canadian cohort study. *Blood*. 2007;109:145–54.

[20] Goodeve A, Eikenboom J, Castaman G, Rodeghiero F, Federici AB, Batlle J, Meyer D, Mazurier C, Goudemand J, Schneppenheim R, Budde U, Ingerslev J, Habart D, Vorlova Z, Holmberg L, Lethagen S, Pasi J, Hill F, Soteh MH, Baronciani L, et al. Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, molecular and clinical markers for the diagnosis and management of type 1 von Willebrand disease (MCMDM-1VWD). *Blood*. 2007;109:112–21.

[21] Lavin M, Aguila S, Schneppenheim S, Dalton N, Jones KL, O'Sullivan JM, O'Connell NM, Ryan K, White B, Byrne M, Rafferty M, Doyle MM, Nolan M, Preston RJS, Budde U, James P, Di Paola J, O'Donnell JS. Novel insights into the clinical phenotype and pathophysiology underlying low VWF levels. *Blood*. 2017;130:2344–53.

[22] Swystun LL, Lillicrap D. Genetic regulation of plasma von Willebrand factor levels in health and disease. *J Thromb Haemost*. 2018;16:2375–90.

[23] Kanaji S, Fahs SA, Shi Q, Haberichter SL, Montgomery RR. Contribution of platelet vs. endothelial VWF to platelet adhesion and hemostasis. *J Thromb Haemost*. 2012;10:1646–52.

[24] Zhou YF, Eng ET, Zhu J, Lu C, Walz T, Springer TA. Sequence and structure relationships within von Willebrand factor. *Blood*. 2012;120:449–58.

[25] Huang RH, Wang Y, Roth R, Yu X, Purvis AR, Heuser JE, Egelman EH, Sadler JE. Assembly of Weibel-Palade body-like tubules from N-terminal domains of von Willebrand factor. *Proc Natl Acad Sci U S A*. 2008;105:482–7.

[26] Berriman JA, Li S, Hewlett LJ, Wasilewski S, Kiskin FN, Carter T, Hannah MJ, Rosenthal PB. Structural organization of Weibel-Palade bodies revealed by cryo-EM of vitrified endothelial cells. *Proc Natl Acad Sci U S A*. 2009;106:17407–12.

[27] Zeng J, Shu Z, Liang Q, Zhang J, Wu W, Wang X, Zhou A. Structural basis of von Willebrand factor multimerization and tubular storage. *Blood*. 2022;139:3314–24.

[28] Weibel ER, Palade GE. New cytoplasmic components in arterial endothelia. *J Cell Biol*. 1964;23:101–12.

[29] Schillemans M, Karampini E, Kat M, Bierings R. Exocytosis of Weibel–Palade bodies: how to unpack a vascular emergency kit. *J Thromb Haemost*. 2019;17:6–18.

[30] Hordijk S, Carter T, Bierings R. A new look at an old body: molecular determinants of Weibel-Palade body composition and von Willebrand factor exocytosis. *J Thromb Haemost*. 2024;22:1290–303.

[31] Kat M, Karampini E, Hoogendijk AJ, Bürgisser PE, Mulder AA, Van Alphen FPJ, Olins J, Geerts D, Van den Biggelaar M, Margadant C, Voorberg J, Bierings R. Syntaxin 5 determines Weibel-Palade body size and von Willebrand factor secretion by controlling Golgi architecture. *Haematologica*. 2022;107:1827–39.

[32] Bierings R, Hellen N, Kiskin N, Knipe L, Fonseca AV, Patel B, Meli A, Rose M, Hannah MJ, Carter T. The interplay between the Rab27A effectors Slp4-a and MyRIP controls hormone-evoked Weibel-Palade body exocytosis. *Blood*. 2012;120:2757–67.

[33] Erent M, Meli A, Moisoi N, Babich V, Hannah MJ, Skehel P, Knipe L, Zupancic G, Ogden D, Carter TD. Rate, extent and concentration dependence of histamine-evoked Weibel-Palade body exocytosis determined from individual fusion events in human endothelial cells. *J Physiol*. 2007;583:195–212.

[34] Hannah MJ, Hume AN, Arribas M, Williams R, Hewlett LJ, Seabra MC, Cutler DF. Weibel-Palade bodies recruit Rab27 by a content-driven, maturation-dependent mechanism that is independent of cell type. *J Cell Sci*. 2003;116:3939–48.

[35] Knop M, Aareskjold E, Bode G, Gerke V. Rab3D and annexin A2 play a role in regulated secretion of vWF, but not tPA, from endothelial cells. *EMBO J*. 2004;23:2982–92.

[36] Kat M, Bürgisser PE, Janssen H, De Cuyper IM, Conte IL, Hume AN, Carter T, Voorberg J, Margadant C, Bierings R. GDP/GTP exchange factor MADD drives activation and recruitment of secretory Rab GTPases to Weibel-Palade bodies. *Blood Adv*. 2021;5:5116–27.

[37] Nightingale TD, Pattni K, Hume AN, Seabra MC, Cutler DF. Rab27a and MyRIP regulate the amount and multimeric state of VWF released from endothelial cells. *Blood*. 2009;113:5010–8.

[38] Terglane J, Menche D, Gerke V. Acidification of endothelial Weibel-Palade bodies is mediated by the vacuolar-type H+-ATPase. *PLoS One*. 2022;17:e0270299. https://doi.org/10.1371/journal.pone.0270299

[39] Van Breevoort D, Snijders AP, Hellen N, Weckhuysen S, van Hooren KWEM, Eikenboom J, Valentijn K, Fernandez-Borja M, Ceulemans B, De Jonghe P, Voorberg J, Hannah M, Carter T, Bierings R. STXBP1 promotes Weibel-Palade body exocytosis through its interaction with the Rab27A effector Slp4-a. *Blood*. 2014;123:3185–94.

[40] Conte IL, Hellen N, Bierings R, Mashanov GI, Manneville JB, Kiskin NI, Hannah MJ, Molloy JE, Carter T. Interaction between MyRIP and the actin cytoskeleton regulates Weibel–Palade body trafficking and exocytosis. *J Cell Sci*. 2016;129:592–603.

[41] Chehab T, Santos NC, Holthenrich A, Koerdt SN, Disse J, Schuberth C, Nazmi AR, Neeft M, Koch H, Man KNM, Wojcik SM, Martin TFJ, van der Sluijs P, Brose N, Gerke V. A novel Munc13-4/S100A10/annexin A2 complex promotes Weibel-Palade body exocytosis in endothelial cells. *Mol Biol Cell*. 2017;28:1688–700.

[42] Schillemans M, Karampini E, van den Eshof BL, Gangaev A, Hofman M, van Breevoort D, Meems H, Janssen H, Mulder AA, Jost CR, Escher JC, Adam R, Carter T, Koster AJ, van den Biggelaar M, Voorberg J, Bierings R. Weibel-Palade body localized

syntaxin-3 modulates von Willebrand factor secretion from endothelial cells. *Arterioscler Thromb Vasc Biol*. 2018;38:1549–61.

[43] Lenzi C, Stevens J, Osborn D, Hannah MJ, Bierings R, Carter T. Synaptotagmin 5 regulates Ca^{2+}-dependent Weibel–Palade body exocytosis in human endothelial cells. *J Cell Sci*. 2019;132: jcs221952. https://doi.org/10.1242/jcs.221952

[44] Francis CR, Claflin S, Kushner EJ. Synaptotagmin-like protein 2a regulates angiogenic lumen formation via Weibel-Palade body apical secretion of angiopoietin-2. *Arterioscler Thromb Vasc Biol*. 2021;41:1972–86.

[45] Holthenrich A, Terglane J, Naß J, Mietkowska M, Kerkhoff E, Gerke V. Spire1 and myosin Vc promote Ca2+-evoked externalization of von Willebrand factor in endothelial cells. *Cell Mol Life Sci*. 2022;79:96. https://doi.org/10.1007/s00018-021-04108-x

[46] Naß J, Koerdt SN, Biesemann A, Chehab T, Yasuda T, Fukuda M, Martín-Belmonte F, Gerke V. Tip-end fusion of a rod-shaped secretory organelle. *Cell Mol Life Sci*. 2022;79:344. https://doi.org/10.1007/s00018-022-04367-2

[47] Smith NL, Chen MH, Dehghan A, Strachan DP, Basu S, Soranzo N, Hayward C, Rudan I, Sabater-Lleal M, Bis JC, de Maat MPM, Rumley A, Kong X, Yang Q, Williams FMK, Vitart V, Campbell H, Mälarstig A, Wiggins KL, Van Duijn CM, et al. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. *Circulation*. 2010;121:1382–92.

[48] Desch KC, Ozel AB, Siemieniak D, Kalish Y, Shavit JA, Thornburg CD, Sharathkumar AA, McHugh CP, Laurie CC, Crenshaw A, Mirel DB, Kim Y, Cropp CD, Molloy AM, Kirke PN, Bailey-Wilson JE, Wilson AF, Mills JL, Scott JM, Brody LC, et al. Linkage analysis identifies a locus for plasma von Willebrand factor undetected by genome-wide association. *Proc Natl Acad Sci U S A*. 2013;110:588–93.

[49] Van Loon J, Dehghan A, Weihong T, Trompet S, McArdle WL, Asselbergs FFW, Chen MH, Lopez LM, Huffman JE, Leebeek FWG, Basu S, Stott DJ, Rumley A, Gansevoort RT, Davies G, Wilson JJF, Witteman JCM, Cao X, de Craen AJM, Bakker SJL, et al. Genome-wide association studies identify genetic loci for low von Willebrand factor levels. *Eur J Hum Genet*. 2016;24:1035–40.

[50] Schillemans M, Karampini E, Hoogendijk AJ, Wahedi M, van Alphen FPJ, van den Biggelaar M, Voorberg J, Bierings R. Interaction networks of Weibel-Palade body regulators syntaxin-3 and syntaxin binding protein 5 in endothelial cells. *J Proteomics*. 2019;205:103417. https://doi.org/10.1016/j.jprot.2019.103417

[51] Hannah MJ, Williams R, Kaur J, Hewlett LJ, Cutler DF. Biogenesis of Weibel–Palade bodies. *Semin Cell Dev Biol*. 2002;13:313–24.

[52] Michaux G, Hewlett LJ, Messenger SL, Goodeve AC, Peake IR, Daly ME, Cutler DF. Analysis of intracellular storage and regulated secretion of 3 von Willebrand disease-causing variants of von Willebrand factor. *Blood*. 2003;102:2452–8.

[53] van den Biggelaar M, Meijer AB, Voorberg J, Mertens K. Intracellular cotrafficking of factor VIII and von Willebrand factor type 2N variants to storage organelles. *Blood*. 2009;113:3102–9.

[54] Wang JW, Valentijn KM, de Boer HC, Dirven RJ, van Zonneveld AJ, Koster AJ, Voorberg J, Reitsma PH, Eikenboom J. Intracellular storage and regulated secretion of von Willebrand factor in quantitative von Willebrand disease. *J Biol Chem*. 2011;286:24180–8.

[55] de Boer S, Laan S, Dirven R, Eikenboom J. Approaches to induce the maturation process of human induced pluripotent stem cell derived-endothelial cells to generate a robust model. *PLoS One*. 2024;19:e0297465. https://doi.org/10.1371/journal.pone.0297465

[56] Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest*. 2000;105:71–7.

[57] Medina RJ, Barber CL, Sabatier F, Dignat-George F, Melero-Martin JM, Khosrotehrani K, Ohneda O, Randi AM, Chan JKY, Yamaguchi T, Van Hinsbergh VWM, Yoder MC, Stitt AW. Endothelial progenitors: a consensus statement on nomenclature. *Stem Cells Transl Med*. 2017;6:1316–20.

[58] Smadja DM. Vasculogenic stem and progenitor cells in human: future cell therapy product or liquid biopsy for vascular disease. *Adv Exp Med Biol*. 2019;1201:215–37.

[59] Zhang Q, Cannavicci A, Kutryk MJB. Exploring endothelial colony-forming cells to better understand the pathophysiology of disease: an updated review. *Stem Cells Int*. 2022;2022:4460041. https://doi.org/10.1155/2022/4460041

[60] Tura O, Skinner EM, Barclay GR, Samuel K, Gallagher RCJ, Brittan M, Hadoke PWF, Newby DE, Turner ML, Mills NL. Late outgrowth endothelial cells resemble mature endothelial cells and are not derived from bone marrow. *Stem Cells*. 2013;31: 338–48.

[61] Lin Y, Banno K, Gil CH, Myslinski J, Hato T, Shelley WC, Gao H, Xuei X, Liu Y, Basile DP, Yoshimoto M, Prasain N, Tarnawsky SP, Adams RH, Naruse K, Yoshida J, Murphy MP, Horie K, Yoder MC. Origin, prospective identification, and function of circulating endothelial colony-forming cells in mice and humans. *JCI Insight*. 2023;8:e164781. https://doi.org/10.1172/jci.insight.164781

[62] Martin-Ramirez J, Hofman M, van den Biggelaar M, Hebbel RP, Voorberg J. Establishment of outgrowth endothelial cells from peripheral blood. *Nat Protoc*. 2012;7:1709–15.

[63] Smadja DM, Melero-Martin JM, Eikenboom J, Bowman M, Sabatier F, Randi AM. Standardization of methods to quantify and culture endothelial colony-forming cells derived from peripheral blood: position paper from the International Society on Thrombosis and Haemostasis SSC. *J Thromb Haemost*. 2019;17:1190–4.

[64] Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood*. 2004;104:2752–60.

[65] van Beem RT, Verloop RE, Kleijer M, Noort WA, Loof N, Koolwijk P, van der Schoot CE, van Hinsbergh VW, Zwaginga JJ. Blood outgrowth endothelial cells from cord blood and peripheral blood: angiogenesis-related characteristics *in vitro*. *J Thromb Haemost*. 2009;7:217–26.

[66] Béland S, Désy O, Bouchard-Boivin F, Gama A, De Serres SA. Endothelial colony forming cells generated from cryopreserved peripheral blood mononuclear cells. *Hum Immunol*. 2021;82: 309–14.

[67] de Jong A, Weijers E, Dirven R, de Boer S, Streur J, Eikenboom J. Variability of von Willebrand factor-related parameters in endothelial colony forming cells. *J Thromb Haemost*. 2019;17: 1544–54.

[68] de Boer S, Bowman M, Notley C, Mo A, Lima P, de Jong A, Dirven R, Weijers E, Lillicrap D, James P, Eikenboom J. Endothelial characteristics in healthy endothelial colony forming cells; generating a robust and valid *ex vivo* model for vascular disease. *J Thromb Haemost*. 2020;18:2721–31.

[69] Laan SNJ, de Boer S, Dirven RJ, van Moort I, Kuipers TB, Mei H, Bierings R, Eikenboom J. SYMPHONY consortium. Transcriptional and functional profiling identifies inflammation and endothelial-to-mesenchymal transition as potential drivers for phenotypic heterogeneity within a cohort of endothelial colony forming cells. *J Thromb Haemost*. 2024;22:2027–38.

[70] Berber E, James PD, Hough C, Lillicrap D. An assessment of the pathogenic significance of the R924Q von Willebrand factor substitution. *J Thromb Haemost*. 2009;7:1672–9.

[71] Starke RD, Paschalaki KE, Dyer CE, Harrison-Lavoie KJ, Cutler JA, McKinnon TA, Millar CM, Cutler DF, Laffan MA, Randi AM. Cellular

and molecular basis of von Willebrand disease: studies on blood outgrowth endothelial cells. *Blood*. 2013;121:2773–84.

[72] Wang JW, Bouwens EAM, Pintao MC, Voorberg J, Safdar H, Valentijn KM, De Boer HC, Mertens K, Reitsma PH, Eikenboom J. Analysis of the storage and secretion of von Willebrand factor in blood outgrowth endothelial cells derived from patients with von Willebrand disease. *Blood*. 2013;121:2762–72.

[73] Bowman ML, Pluthero FG, Tuttle A, Casey L, Li L, Christensen H, Robinson KS, Lillicrap D, Kahr WHA, James P. Discrepant platelet and plasma von Willebrand factor in von Willebrand disease patients with p.Pro2808Leufs*24. *J Thromb Haemost*. 2017;15:1403–11.

[74] Bowman M, Casey L, Selvam SN, Lima PDA, Rawley O, Hinds M, Tuttle A, Grabell J, Iorio A, Walker I, Lillicrap D, James P. von Willebrand factor propeptide variants lead to impaired storage and ER retention in patient-derived endothelial colony-forming cells. *J Thromb Haemost*. 2022;20:1599–609.

[75] Okamoto S, Tamura S, Sanda N, Odaira K, Hayakawa Y, Mukaide M, Suzuki A, Kanematsu T, Hayakawa F, Katsumi A, Kiyoi H, Kojima T, Matsushita T, Suzuki N. VWF-Gly2752Ser, a novel non-cysteine substitution variant in the CK domain, exhibits severe secretory impairment by hampering C-terminal dimer formation. *J Thromb Haemost*. 2022;20:1784–96.

[76] Yadegari H, Jamil MA, Müller J, Marquardt N, Rawley O, Budde U, El-Maarri O, Lillicrap D, Oldenburg J. Multifaceted pathomolecular mechanism of a VWF large deletion involved in the pathogenesis of severe VWD. *Blood Adv*. 2022;6:1038–53.

[77] Kat M, Margadant C, Voorberg J, Bierings R. Dispatch and delivery at the ER–Golgi interface: how endothelial cells tune their hemostatic response. *FEBS J*. 2022;289:6863–70.

[78] Othman M, Chirinian Y, Brown C, Notley C, Hickson N, Hampshire D, Buckley S, Waddington S, Parker AL, Baker A, James P, Lillicrap D. Functional characterization of a 13-bp deletion (c.-1522_-1510del13) in the promoter of the von Willebrand factor gene in type 1 von Willebrand disease. *Blood*. 2010;116:3645–52.

[79] Hawke L, Bowman ML, Poon MC, Scully MF, Rivard GE, James PD. Characterization of aberrant splicing of von Willebrand factor in von Willebrand disease: an underrecognized mechanism. *Blood*. 2016;128:584–93.

[80] Yadegari H, Biswas A, Akhter MS, Driesen J, Ivaskevicius V, Marquardt N, Oldenburg J. Intron retention resulting from a silent mutation in the VWF gene that structurally influences the 5′ splice site. *Blood*. 2016;128:2144–52.

[81] Yadegari H, Jamil MA, Marquardt N, Oldenburg J. A homozygous deep intronic variant causes von Willebrand factor deficiency and lack of endothelial-specific secretory organelles, Weibel–Palade bodies. *Int J Mol Sci*. 2022;23:3095. https://doi.org/10.3390/ijms23063095

[82] Ng CJ, Liu A, Venkataraman S, Ashworth KJ, Baker CD, O'Rourke R, Vibhakar R, Jones KL, Di Paola J. Single-cell transcriptional analysis of human endothelial colony-forming cells from patients with low VWF levels. *Blood*. 2022;139:2240–51.

[83] Kloosterman R, Zago-Schmitt M, Grabell J, Thibeault L, De Lima PA, Bowman M, Tyryshkin K, Hindmarch CCT, Renwick N, James P. A transcriptome analysis of basal and stimulated VWF release from endothelial cells derived from patients with type 1 VWD. *Blood Adv*. 2023;7:1477–87.

[84] De Meyer SF, Vanhoorelbeke K, Chuah MK, Pareyn I, Gillijns V, Hebbel RP, Collen D, Deckmyn H, VandenDriessche T. Phenotypic correction of von Willebrand disease type 3 blood-derived endothelial cells with lentiviral vectors expressing von Willebrand factor. *Blood*. 2006;107:4728–36.

[85] de Jong A, Dirven RJ, Boender J, Atiq F, Anvar SY, Leebeek FWG, Van Vlijmen BJM, Eikenboom J. *Ex vivo* Improvement of a von Willebrand disease type 2A phenotype using an allele-specific small-interfering RNA. *Thromb Haemost*. 2020;120:1569–79.

[86] Randi AM, Smith KE, Castaman G. von Willebrand factor regulation of blood vessel formation. *Blood*. 2018;132:132–40.

[87] Makris M, Federici AB, Mannucci PM, Bolton-Maggs PHB, Yee TT, Abshire T, Berntorp E. The natural history of occult or angiodysplastic gastrointestinal bleeding in von Willebrand disease. *Haemophilia*. 2015;21:338–42.

[88] Ocran E, Chornenki NLJ, Bowman M, Sholzberg M, James P. Gastrointestinal bleeding in von Willebrand patients: special diagnostic and management considerations. *Expert Rev Hematol*. 2023;16:575–84.

[89] Lorenzi O, Frieden M, Villemin P, Fournier M, Foti M, Vischer UM. Protein kinase C-delta mediates von Willebrand factor secretion from endothelial cells in response to vascular endothelial growth factor (VEGF) but not histamine. *J Thromb Haemost*. 2008;6:1962–9.

[90] Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, Kriz W, Thurston G, Augustin HG. The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood*. 2004;103:4150–6.

[91] van Breevoort D, van Agtmaal EL, Dragt BS, Gebbinck JK, Dienava-Verdoold I, Kragt A, Bierings R, Horrevoets AJ, Valentijn KM, Eikenboom JC, Fernandez-Borja M, Meijer AB, Voorberg J. Proteomic screen identifies IGFBP7 as a novel component of endothelial cell-specific Weibel-Palade bodies. *J Proteome Res*. 2012;11:2925–36.

[92] Pergolizzi M, Bizzozero L, Riccitelli E, Pascal D, Samarelli AV, Bussolino F, Arese M. Modulation of angiopoietin 2 release from endothelial cells and angiogenesis by the synaptic protein neuroligin 2. *Biochem Biophys Res Commun*. 2018;501:165–71.

[93] Cossutta M, Darche M, Carpentier G, Houppe C, Ponzo M, Raineri F, Vallée B, Gilles M-E, Villain D, Picard E, Casari C, Denis C, Paques M, Courty J, Cascone I. Weibel-Palade bodies orchestrate pericytes during angiogenesis. *Arterioscler Thromb Vasc Biol*. 2019;39:1843–58.

[94] Somanath PR, Malinin NL, Byzova TV. Cooperation between integrin alphavbeta3 and VEGFR2 in angiogenesis. *Angiogenesis*. 2009;12:177–85.

[95] Ishihara J, Ishihara A, Starke RD, Peghaire CR, Smith KE, McKinnon TAJ, Tabata Y, Sasaki K, White MJV, Fukunaga K, Laffan MA, Lutolf MP, Randi AM, Hubbell JA. The heparin binding domain of von Willebrand factor binds to growth factors and promotes angiogenesis in wound healing. *Blood*. 2019;133:2559–69.

[96] Mobayen G, Smith K, Ediriwickrema K, Starke RD, Solomonidis EG, Laffan MA, Randi AM, McKinnon TAJ. von Willebrand factor binds to angiopoietin-2 within endothelial cells and after release from Weibel–Palade bodies. *J Thromb Haemost*. 2023;21:1802–12.

[97] Texier A, Lenting PJ, Denis CV, Roullet S, Christophe OD. Angiopoietin-2 binds to multiple interactive sites within von Willebrand factor. *Res Pract Thromb Haemost*. 2023;7:102204. https://doi.org/10.1016/j.rpth.2023.102204

[98] Starke RD, Ferraro F, Paschalaki KE, Dryden NH, McKinnon TA, Sutton RE, Payne EM, Haskard DO, Hughes AD, Cutler DF, Laffan MA, Randi AM. Endothelial von Willebrand factor regulates angiogenesis. *Blood*. 2011;117:1071–80.

[99] Groeneveld DJ, van Bekkum T, Dirven RJ, Wang JW, Voorberg J, Reitsma PH, Eikenboom J. Angiogenic characteristics of blood outgrowth endothelial cells from patients with von Willebrand disease. *J Thromb Haemost*. 2015;13:1854–66.

[100] Selvam SN, Casey LJ, Bowman ML, Hawke LG, Longmore AJ, Mewburn J, Ormiston ML, Archer SL, Maurice DH, James P.

Abnormal angiogenesis in blood outgrowth endothelial cells derived from von Willebrand disease patients. *Blood Coagul Fibrinolysis*. 2017;28:521–33.

[101] Schillemans M, Kat M, Westeneng J, Gangaev A, Hofman M, Nota B, van Alphen FPJ, de Boer M, van den Biggelaar M, Margadant C, Voorberg J, Bierings R. Alternative trafficking of Weibel-Palade body proteins in CRISPR/Cas9-engineered von Willebrand factor-deficient blood outgrowth endothelial cells. *Res Pract Thromb Haemost*. 2019;3:718–32.

[102] Wiegerinck CL, Janecke AR, Schneeberger K, Vogel GF, van Haaften-Visser DY, Escher JC, Adam R, Thöni CE, Pfaller K, Jordan AJ, Weis CA, Nijman IJ, Monroe GR, van Hasselt PM, Cutz E, Klumperman J, Clevers H, Nieuwenhuis EES, Houwen RHJ, van Haaften G, et al. Loss of syntaxin 3 causes variant microvillus inclusion disease. *Gastroenterology*. 2014;147:65–8.e10.

[103] Karampini E, Bierings R, Voorberg J. Orchestration of primary hemostasis by platelet and endothelial lysosome-related organelles. *Arterioscler Thromb Vasc Biol*. 2020;40:1441–53.

[104] Karampini E, Schillemans M, Hofman M, van Alphen F, de Boer M, Kuijpers TW, van den Biggelaar M, Voorberg J, Bierings R. Defective AP-3-dependent VAMP8 trafficking impairs Weibel-Palade body exocytosis in Hermansky-Pudlak syndrome type 2 blood outgrowth endothelial cells. *Haematologica*. 2019;104:2091–9.

[105] Kat M, van Moort I, Bürgisser PE, Kuijpers TW, Hofman M, Favier M, Favier R, Margadant C, Voorberg J, Bierings R. Mutations in neurobeachin-like 2 do not impact Weibel-Palade body biogenesis and von Willebrand factor secretion in gray platelet syndrome endothelial colony forming cells. *Res Pract Thromb Haemost*. 2023;7:100086. https://doi.org/10.1016/j.rpth.2023.100086

[106] Cnossen MH, van Moort I, Reitsma SH, de Maat MPM, Schutgens REG, Urbanus RT, Lingsma HF, Mathot RAA, Gouw SC, Meijer K, Bredenoord AL, van der Graaf R, Fijnvandraat K, Meijer AB, van den Akker E, Bierings R, Eikenboom JCJ, van den Biggelaar M, de Haas M, Voorberg J, et al. SYMPHONY consortium: orchestrating personalized treatment for patients with bleeding disorders. *J Thromb Haemost*. 2022;20:2001–11.