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Hemodynamic Disruption Triggers Glomerular Barrier Injury via Endothelial Glycocalyx Degradation in Nephrotic Syndrome

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Background: Nephrotic syndrome (NS) is characterized by severe proteinuria, generalized edema and a hypercoagulable state. The clinical manifestations of NS are well recognized, yet the mechanistic link between renal microhemodynamic disturbance and glomerular filtration barrier (GFB) injury remains poorly defined. Endothelial glycocalyx (EG) serves as a critical vascular mechanosensor and barrier regulator, and its degradation contributes to microcirculatory dysfunction and increased vascular permeability in renal diseases. **Methods:** This study established an integrated platform consisting of an adriamycin (ADR)-induced NS rat model and microphysiological vascular-on-a-chip (VOAC) and glomerulus-on-a-chip (GOAC) systems. In vivo, hemorheological parameters, coagulation function, EG biomarker expression and urinary metabolites were detected, and the therapeutic efficacy of sulodexide was evaluated in NS rats. In vitro, controlled laminar shear stress was applied in VOAC and GOAC models to explore the independent effects of mechanical stimulation on endothelial integrity and GFB permeability. **Results:** ADR-induced NS rats presented prominent hemorheological abnormalities and hypercoagulability, accompanied by significant EG damage characterized by decreased expression of EG-related proteins and increased urinary excretion of glycocalyx metabolites. To further clarify the underlying mechanical mechanism, in vitro VOAC and GOAC models were utilized, and the results confirmed that low

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shear stress triggered EG shedding, cytoskeletal disorganization, elevated adhesion molecule expression, and eventually increased GFB permeability in a dose-dependent manner. Furthermore, in vivo intervention experiments demonstrated that sulodexide treatment could effectively protect EG structural integrity, ameliorate hemorheological disorders, and restore impaired microvascular barrier function in NS rats. **Conclusions:**EG degradation critically bridges hemodynamic alterations and GFB injury in NS. Organ-on-a-chip systems provide a robust platform for elucidating shear stress-mediated renal barrier dysfunction. This study reveals a plausible pathogenic mechanism of NS and indicates the potential of EG-targeted sulodexide therapy for NS treatment.

Keywords: Organ-on-a-chip; Endothelial glycocalyx; Fluid shear stress; Nephrotic syndrome; Glomerular filtration barrier

Introduction

Organ-on-a-chip (OOC) technology has rapidly emerged as a transformative platform in biomedicine. It was recognized as one of the Ten Emerging Technologies at the World Economic Forum in Davos in 2016. These microfluidic devices mimic organ-level functions within microscale bioreactors. As a multidisciplinary field integrating physics, engineering, chemistry, and biology, microfluidics enables precise manipulation of minute fluid volumes, thereby facilitating high-resolution analysis of cellular and tissue functions on a chip^[1-3]. However, prevailing OOC platforms lack essential multiscale architectures-specifically the integration of vascular endothelium and basement membrane-and often fail to provide critical physiological mechanical cues, such as tension, pressure, and fluid shear stress, which are indispensable for proper organogenesis and function^[4]. Recent advances in microfluidic engineering are addressing these limitations. By integrating in vitro cell culture with microstructured flow environments, OOC-also termed “tissue chips” or “microphysiological systems”-can recapitulate complex tissue architecture and physiological functions ex vivo. This approach enables the reconstruction of organ microenvironments, incorporation of multiple cell types, replication of extracellular

matrix features, and precise control over biomechanical and biochemical parameters^[5,6]. The traditional Chinese medicine (TCM) theory of “Unsmooth blood circulation (UBC) results in water retention (WR)” (UBC→WR) offers a profound perspective on the interdependence between blood flow and fluid balance. According to this theory, impaired blood circulation disrupts water metabolism, ultimately leading to edema. Here, “UBC” refers to various forms of blood stasis, whereas “WR” describes the pathological disruption of fluid homeostasis, typically manifesting as interstitial fluid accumulation within skin and mucous membranes. Notably, the clinical presentation of NS aligns with this ancient concept. NS, a chronic kidney disease, is characterized by increased permeability of the GFB, leading to substantial urinary protein loss. Furthermore, modern clinical studies have established that a hypercoagulable state in NS significantly increases the risk of thromboembolic events, including deep vein thrombosis, arterial thrombosis, and pulmonary embolism^[7-9]. This hypercoagulable state frequently coincides with proteinuria and edema, mirroring the core pathophysiological features described by the UBC→WR theory. Therefore, elucidating the mechanistic link between hemodynamic dysfunction and fluid retention in NS presents a valuable opportunity to integrate traditional TCM concepts with contemporary biomedical science.

Proteinuria is the hallmark clinical manifestation of NS and an independent risk factor for progression to renal failure^[10-12]. Understanding its underlying mechanisms and identifying therapeutic targets for proteinuric patients are therefore clinical priorities^[13]. Current research predominantly focuses on podocytes^[14-16], yet proteinuria can still occur when podocyte foot processes are structurally preserved^[17-18], suggesting that other GFB components are critically involved^[19-21]. The glomerular endothelium, as the first filtration interface, plays a key role in vascular permeability regulation. Its luminal surface is coated by the EG, a dynamic, hydrated mesh of membrane-bound proteoglycans and glycoproteins^[22]. Proteoglycans, with their glycosaminoglycan side chains, form the EG “scaffold” and hydrophilic gel-like structure that retains water and maintains its stability^[23]. While EG degradation can be triggered by hyperglycemia and inflammation^[24,25], but the

86 impact of blood flow conditions on EG integrity remains insufficiently studied.
87 Evidence suggests that alterations in flow patterns-such as those occurring in
88 extracorporeal circulation-can produce shear stress fluctuations, potentially
89 contributing to acute EG injury. However, the fragility and nanoscale complexity of
90 the EG render it difficult to visualize and quantify directly. Existing approaches,
91 including in vivo, ex vivo, and in vitro models, have methodological limitations. In
92 vivo estimation often relies on indirect tracer-based volume distribution
93 measurements, inferring EG thickness from the differential distribution of permeable
94 versus impermeable markers. While in vitro endothelial cell cultures allow precise
95 environmental control, ex vivo setups fail to reproduce physiological shear forces and
96 microvascular complexity^[26-28]. Consequently, accurately characterizing EG structural
97 and functional dynamics under physiological and pathological shear conditions
98 remains a major research challenge.

99 To address these challenges, we adopted an integrated approach combining in vivo
100 and microfluidic strategies. Using an ADR-induced NS rat model, we integrated
101 microfluidic OOC platforms with conventional animal experiments to investigate EG
102 abnormalities in conditions associated with blood stasis and water retention.
103 Specifically, we utilized a VOAC to evaluate the impact of defined FSS on EG
104 structural integrity, and a dual-layer GOAC to assess the functional consequences of
105 EG alteration on GFB permeability. Through correlative morphological and functional
106 analyses, we aim to reinterpret the TCM principle of “UBC→WR” from the lens of
107 EG mechanobiology. This study not only demonstrates the utility of OOC technology
108 as an innovative framework for bridging TCM theory with modern biomedical
109 science but also provides new insights into the physiological simulation of organ
110 microenvironments, the biological basis of traditional concepts, and the integration of
111 Eastern medicine with advanced technology (Fig.1).

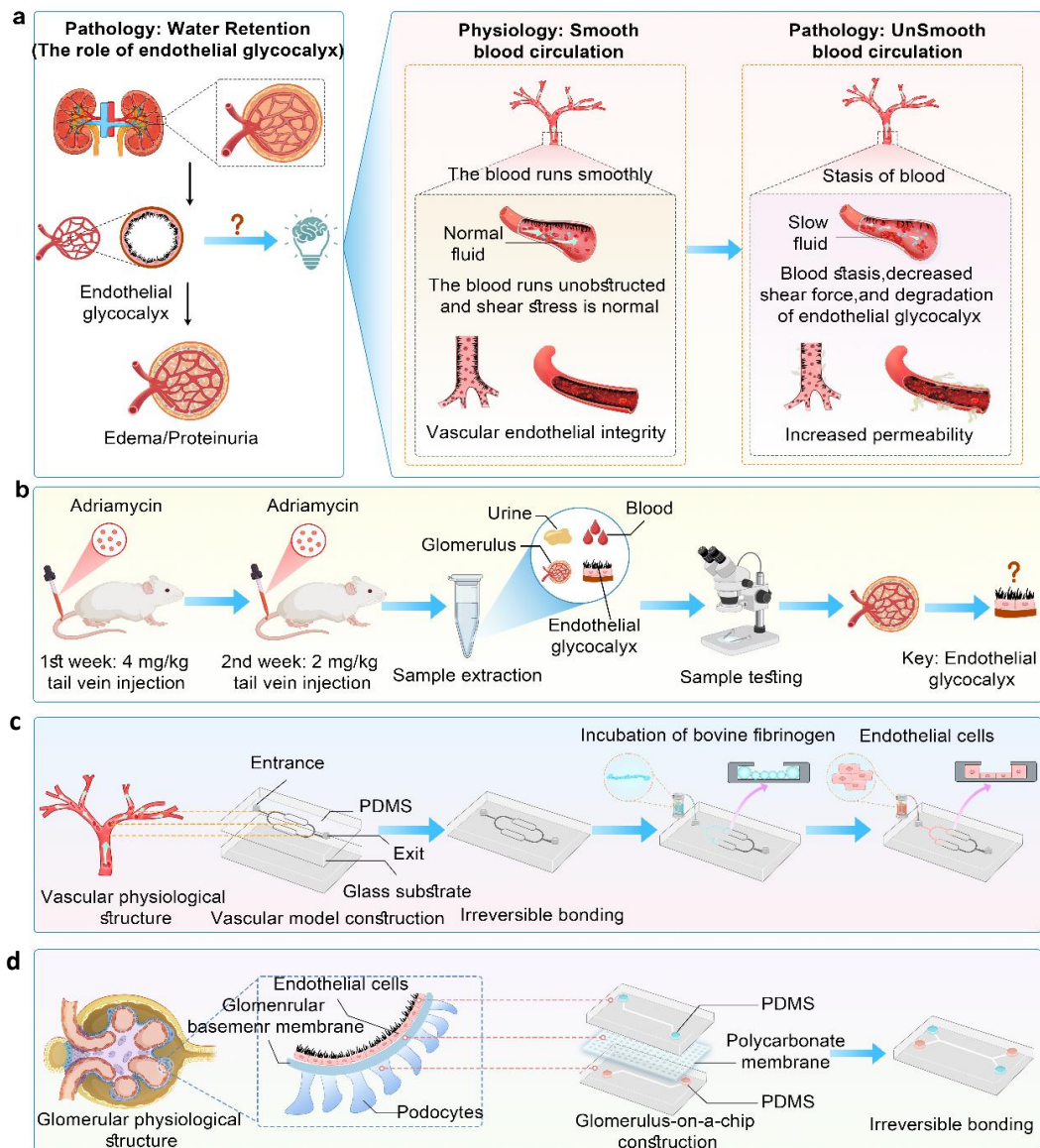


Fig.1. Conceptual framework and experimental platforms for investigating the EG in NS. (a) Pathophysiological concept. Normal blood flow maintains vascular integrity via physiological shear stress, whereas blood stasis reduces shear force, causing EG loss, increased permeability, proteinuria, and edema. (b) In vivo NS model. Rats received adriamycin injections (week 1: 4 mg/kg; week 2: 2 mg/kg), followed by collection of urine, blood, and glomerular tissue for EG analysis.(c) VOAC fabrication. PDMS microchannels, patterned on vascular geometry, were irreversibly bonded to glass and seeded with endothelial cells to model EG formation. (d) GOAC fabrication. Two PDMS layers separated by a porous polycarbonate membrane were seeded with endothelial cells (upper) and podocytes (lower) to mimic the GFB.

Methods

Establishment of animal models

A total of 150 male Sprague-Dawley rats (SPF grade; 8 weeks old; 300 ± 50 g) were obtained under the certification SCXK (Liao) 2020-0001. All animals were confirmed negative for proteinuria by 2 consecutive qualitative urine protein tests (urine protein dipstick). Experimental procedures were approved by the Animal Ethics Committee of Anhui University of Chinese Medicine (Ethics Number: AHUCM-rats-2023052). Rats were housed under standard laboratory conditions at $25 \pm 2^{\circ}\text{C}$ and $50 \pm 10\%$ relative humidity, with a 12-hour light/dark cycle and free access to food and water. After 1 week of acclimatization, 30 rats were randomly assigned to the control group (Control), and the remaining 120 rats received ADR via tail-vein injection over 2 consecutive weeks (Week 1: 4 mg/kg; Week 2: 2 mg/kg) to induce the NS model. 24 h urine samples were collected 2 weeks after model induction; rats with urinary protein excretion ≥ 100 mg/24 h were considered successfully modeled^[29]. These modeled animals were then randomly divided into 3 groups: untreated model (Model), prednisone acetate-treated (M+Pa), and sulodexide-treated (M+S). Control and Model groups received saline via oral gavage, whereas M+Pa and M+S were administered prednisone acetate (10 mg/kg/day) and sulodexide (15 mg/kg/day), respectively. All treatments continued for 3 weeks. Rats were euthanized at predefined time points: prior to modeling, at 1 week and 2 weeks post-modeling, and at 1, 2, and 3 weeks after the initiation of drug administration. Blood and kidney tissues were collected immediately post-mortem. For tissue collection, rats were anesthetized with sodium pentobarbital. The abdominal cavity was opened, and blood was drawn from the abdominal aorta. The right kidney was excised, the renal capsule removed, and the tissue bisected: one half was fixed in 4% paraformaldehyde, and the other stored in a cryovial at -80°C for subsequent analyses. The left kidney was subjected to in situ perfusion: 5 mL of 2% lanthanum nitrate solution was perfused via the renal artery, followed by 5 mL of 2% lanthanum nitrate-alginate fixative. Once the kidney cortex turned white, $\sim 1\text{ mm}^3$ of cortical tissue was excised and fixed in 2% lanthanum

nitrate-alginate solution for ultrastructural analysis.

Reagent preparation

ADR was purchased from Zhejiang Hai Zheng Pharmaceutical Co., Ltd. (China); prednisone acetate tablets from Zhejiang Xian Ju Pharmaceutical Co., Ltd. (China); sulodexide soft capsules from Alpha Sigma Corporation (Italy); lanthanum nitrate hydrate from BBI Life Sciences (China); sodium dimethylarsenate from Xiya Chemical Technology (Shandong) Co., Ltd. (China); HA ELISA detection kit and HS ELISA kit from Shanghai Keai Bo Biological Technology Co., Ltd. (China); BCA protein concentration assay kit from Biosharp (China); VCAM-1 and ICAM-1 antibodies from Abcam, Inc. (UK); Syndecan-1, Glypican-1, and CD44 antibodies from Proteintech (USA); wheat germ agglutinin (WGA) and phalloidin from Thermo Fisher Scientific (USA).

Reagent configuration^[30]: 1. 0.2 mol/L sodium dimethylarsenate-HCl buffer: mix 25 mL of 0.2 mol/L sodium dimethylarsenate with 2.1 mL of 0.2 mol/L HCl, then add 72.9 mL of deionized water. 2. 2% lanthanum nitrate solution (100 mL): dissolve 2 g of lanthanum nitrate in 100 mL of 0.2 mol/L sodium dimethylarsenate-HCl buffer, and adjust the pH to 7.1. 3. 2% lanthanum nitrate-glutaraldehyde fixative (100 mL): mix 2 g of lanthanum nitrate, 5 mL of 50% glutaraldehyde solution, and 2 g of polyformaldehyde in 95 mL of 0.2 mol/L sodium dimethylarsenate-HCl buffer, and adjust the pH to 7.2.

Chip design

The VOAC consisted of 2 layers: a glass slide forming the bottom layer and a polydimethylsiloxane (PDMS) layer on top, engineered to mimic the human vascular network, including microarteries, capillaries, and microveins. The PDMS channel pattern was based on human vascular geometry and featured a bifurcating architecture with varied widths (600 μm and 300 μm) and a uniform depth of 150 μm . The central observation region corresponded to the capillary-mimicking section with a width of 300 μm . The GOAC was designed to replicate the human GFB and was structured as a 3-layer system. The top and bottom layers were fabricated from PDMS, while the middle layer consisted of a porous polycarbonate membrane acting as an analog of the

glomerular basement membrane. Both the upper (vascular) and lower (urinary) channels were 300 μm wide and 150 μm deep. The polycarbonate membrane had a pore size of 10 μm , a thickness of 16 μm , and a porosity of 5-20%. To maintain a stable fluidic microenvironment, the chips were connected to an Elveflow microfluidic flow control system (Elveflow, France), which utilized an air-pressure pump for precise regulation of flow rates. For real-time visualization and monitoring of cells, the assembled chips were placed within a live-cell imaging workstation equipped with an environmental chamber and a continuous cell-tracking system (Fig. 2).

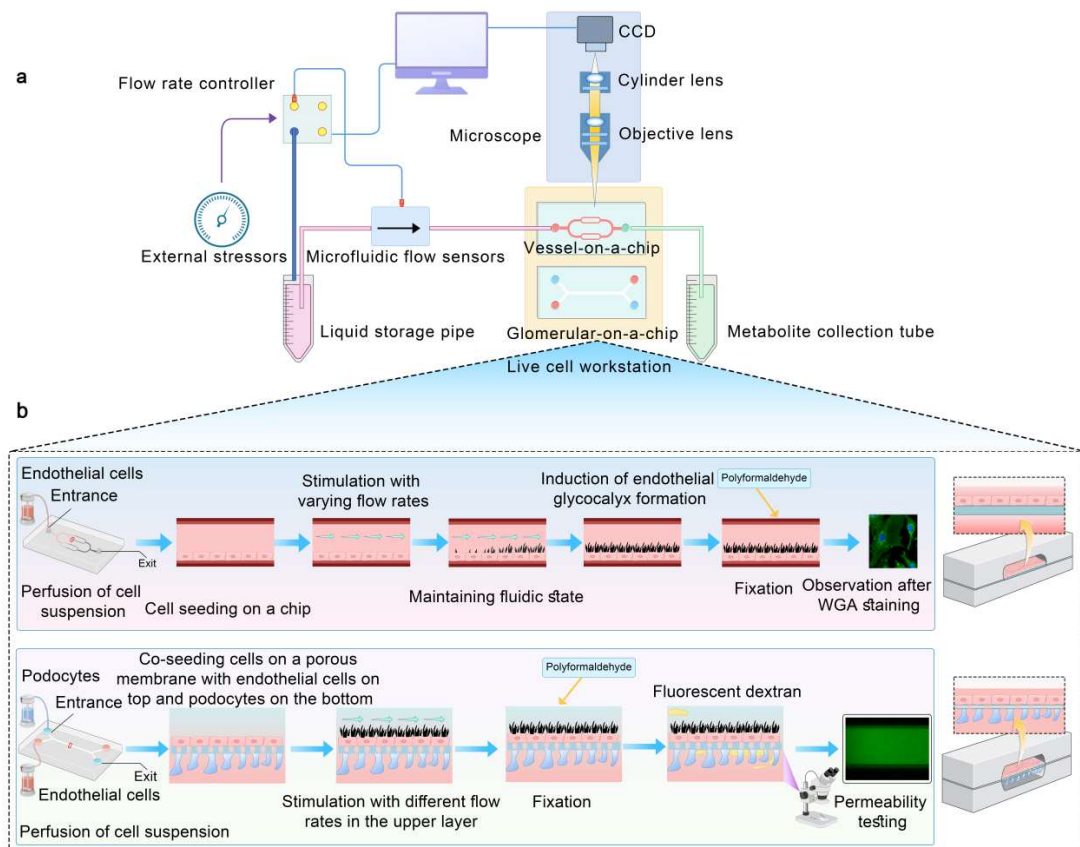


Fig.2. Microfluidic OOC platforms for simulation and analysis of EG function. (a) Microfluidic system setup. Flow rates within the chip were precisely regulated by an externally controlled pressure-driven system, with intrachip conditions continuously monitored via flow sensors. Real-time cellular imaging was conducted in a live-cell workstation equipped with an inverted microscope, CCD camera, and objective lens, enabling dynamic observation of EG under defined shear stress levels. (b) VOAC workflow. A confluent monolayer of endothelial cells was established within the microchannel and

exposed to varying flow rates to assess the effects of fluid shear stress on cellular morphology and EG integrity. EG expression was analyzed by immunofluorescence following WGA labeling. (c) GOAC workflow. HUVECs were seeded into the upper vascular channel and human podocytes into the lower urinary channel, separated by a porous polycarbonate membrane. Differential flow rates were applied to the upper channel to alter EG states, and glomerular permeability was quantified by perfusing fluorescent dextran molecules of defined molecular weights through the vascular compartment, followed by fluorescence intensity measurement.

Chip preparation

A master silicon wafer template was fabricated by spin-coating SU-8 photoresist onto a 4-inch single-side polished silicon wafer, followed by photolithography according to the designed pattern. The PDMS prepolymer was prepared by thoroughly mixing the PDMS base with a curing agent at a 10:1 (w/w) ratio and pouring the mixture onto the chip template. Air bubbles were removed in a vacuum desiccator for 30 min, after which the samples were cured in an oven at 85 °C for 1 h. The cured PDMS layer was cut from the template with a scalpel and perforated using a biopsy punch. The VOAC was assembled by bonding the top PDMS layer to a glass slide via oxygen plasma treatment. A trimmed polycarbonate membrane was bonded to the upper PDMS layer, and the resulting PDMS-polycarbonate assembly was bonded to the lower PDMS layer to obtain the GOAC. All assembled chips were post-baked at 80 °C for 1 hour to strengthen the interfacial bonds. Finally, the chips were sterilized under ultraviolet (UV) light for 40 minutes prior to cell culture experiments.

Calculation of FSS

The fluid used to generate shear stress in this study was primarily complete culture medium, with a density of 1000 kg/m³ and a dynamic viscosity of 0.7 mPa·s^[31]. The medium was modeled as an incompressible, uniformly flowing Newtonian fluid. According to the principle of momentum conservation, the fluid motion within the flow field was governed by the Navier-Stokes equations as follows:

$$\rho \frac{\partial u}{\partial t} + \rho(u \cdot \nabla)u = -\nabla p + \mu \nabla^2 u - \frac{2}{3} \nabla(\mu \nabla \cdot u) + \rho f_v \quad (1.1)$$

Laminar and turbulent flow are the two most common flow regimes encountered in viscous fluids. Laminar flow is characterized by smooth, orderly motion at relatively low velocities, whereas turbulent flow exhibits chaotic, irregular motion at higher velocities. The flow regime can be determined by calculating the dimensionless Reynolds number (Re), which is defined as:

$$Re = \frac{\rho \mu d}{u} \quad (1.2)$$

Because the cells in the microfluidic chip channel grew adherently and had an average height of approximately 2-3 μm , the shear stress experienced by the cells could be approximated by the wall shear stress within the channel. In this study, all reported shear forces refer to wall shear stress. Unlike an idealized, infinitely wide parallel-plate geometry, the microfluidic channel in this system had a rectangular cross-section with a width of 300 μm and a height of 150 μm . The wall shear stress generated in such a channel was calculated using the following equation^[32]:

$$\tau = \frac{6Q\mu}{h^2 w} \quad (1.3)$$

where Q is the volumetric flow rate through the channel(m^3/s), h is the channel height (m), and w is the channel width (m).

Cell preparation

HUVECs and podocytes were purchased from Cyagen (Shanghai) Biotechnologies, Inc. (lot number: iCell-h110/iCell-m081). HUVECs were cultured in ECM supplemented with 5% foetal bovine serum, 1% penicillin-streptomycin, and 1% ECGS (ECM; ScienCell, USA). Podocytes were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin (Gibco Life Technologies, USA). All cell lines were cultured in a cell incubator at 37 °C and 5% CO₂.

Effect of flow rate on the EG

Prior to cell seeding, the microfluidic channels were coated by incubating with a 5

mg/mL bovine fibrinogen solution overnight at 4 °C to promote cell adhesion. The following day, the fibrinogen solution was aspirated, and the channels were gently rinsed with fresh ECM. Cells were then perfused into the channel at a density of 3×10^6 /mL. To prevent medium evaporation during subsequent static culture, the inlet and outlet ports were sealed with sterile pipette tips. The cell-seeded chips were maintained in a standard cell culture incubator (37 °C, 5% CO₂) for 48 hours to allow for cell attachment and monolayer formation, with the culture medium being replaced every 24 h. After this initial static culture period, which ensured stable endothelial monolayer formation, the chips were connected to the microfluidic flow control system. The endothelial cells were subjected to defined fluid flow at four different volumetric flow rates: 2 µL/min, 1 µL/min, 0.5 µL/min, and 0 µL/min (static conditions). Each flow rate was applied for a duration of 24 hours. Following this flow exposure period, the cells within the chips were assessed for subsequent analyses. The parameters examined included endothelial cell morphology, integrity of the EG, organization of the actin cytoskeleton, and expression levels of vascular adhesion molecules.

Transmission electron microscopy (TEM) detection

Two pieces of renal cortical tissue, each approximately 1 mm³ in volume, were immersed in 2% lanthanum nitrate-glutaraldehyde fixative for 24 h. Following fixation, the tissues were rinsed in phosphate-buffered saline (PBS) for 1-6 h, post-fixed in 1% osmium tetroxide for 1-2 h, and subsequently subjected to graded ethanol dehydration. The samples were then infiltrated, embedded, and sectioned at a thickness of 70 nm using an ultramicrotome. Ultrathin sections were stained with 2% uranyl acetate in saturated ethanol and lead citrate for 15 min each, and examined by TEM. Each experimental group consisted of 3 rats.

Western blot assay

Renal cortical tissue was lysed in SDS lysis buffer supplemented with protease inhibitors, and total protein was extracted. Protein concentration was determined using a BCA protein assay kit. Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes, which were subsequently

blocked with BSA. The membranes were incubated with appropriately diluted primary antibodies overnight at 4 °C, washed with PBST, and then incubated with secondary antibodies for 90 min. After three washes in PBST, protein bands were visualized using enhanced chemiluminescence (ECL), and the band intensities were quantified using ImageJ software.

Immunofluorescence detection

Following the chip experiment, the microchannels were washed three times with PBS and fixed. Residual paraformaldehyde was gently removed to avoid detaching cells from the channel walls. The channels were then permeabilized with 0.2% Triton X-100 solution for 20 min, followed by blocking with goat serum working solution for 30 min at room temperature. After washing with PBST for approximately 10 min, the chips were incubated with primary antibodies overnight at 4 °C, washed again with PBST, and subsequently incubated with secondary antibodies for 1 h at room temperature. For cytoskeletal and nuclear staining, the chips were washed with PBS, fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100 for 20 min, and stained with phalloidin-FITC for 30 min at room temperature. Nuclei were counterstained with DAPI for approximately 10 min. Prior to imaging, the chips were rinsed with PBST to remove unbound dye. Fluorescence images were acquired using an inverted fluorescence microscope.

Data analysis

ImageJ software was used to quantify the fluorescence intensity in each image and calculate the average fluorescence intensity. GraphPad Prism software was employed for statistical analyses. Data were expressed as the mean $\pm$ standard deviation (SD). Statistical significance was assessed using one-way analysis of variance (ANOVA) or a two-tailed unpaired Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

Results

Changes in coagulation function and hemorheology during modelling and administration

Analysis of coagulation function showed that, compared with the Control group, PT and APTT were significantly shorter, while FBG levels were significantly higher at 2 weeks after modelling ($P < 0.01$). Relative to the Model group, PT and APTT were significantly prolonged and FBG levels markedly reduced in the M+S group ($P < 0.01$). No significant differences in coagulation parameters were observed between the M+Pa group and the Model group. In the ADR-induced rat model, whole-blood viscosity (middle-cut and high-cut), plasma viscosity, and the red blood cell (RBC) aggregation index were all significantly increased compared with the Control group at 2 weeks after modelling ($P < 0.05$; $P < 0.01$). Compared with the Model group, whole-blood viscosity and RBC aggregation index in the M+S group decreased significantly after 2 weeks of administration, and plasma viscosity was significantly reduced after 3 weeks. In contrast, the M+Pa group showed no significant improvement in whole-blood viscosity, plasma viscosity, or red blood cell-related indices (Fig. 3).

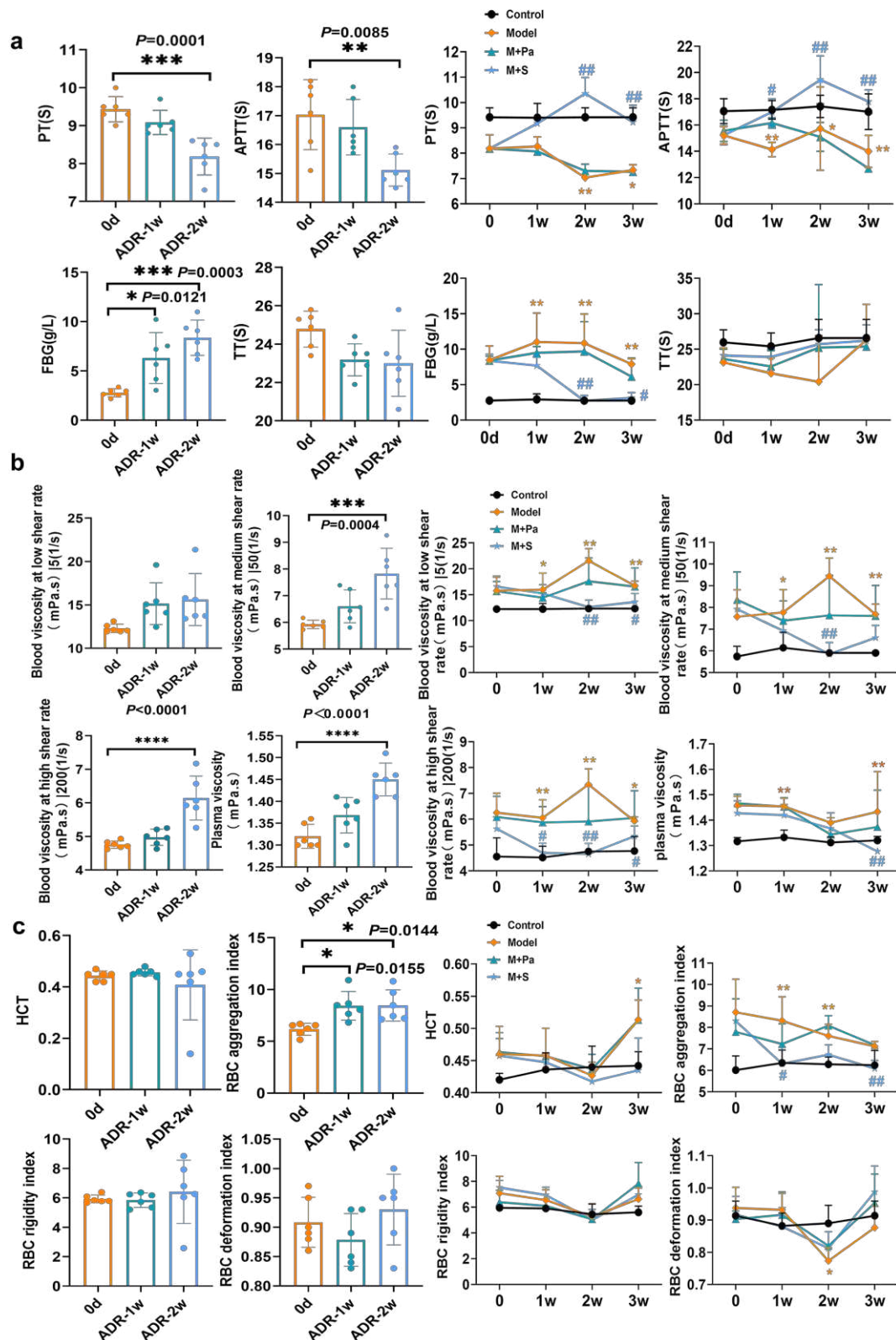


Fig.3. Changes in coagulation function and hemorheological parameters during model establishment and drug administration in rats. (a) Coagulation profiles. Bar plots represent PT, APTT, and FBG levels during model induction; line plots show their temporal changes following drug administration (n=6). (b) Whole-blood viscosity. Bar plots indicate values at

low, medium, and high shear rates during modeling; line plots depict changes across treatment weeks (n=6). (c) Erythrocyte parameters. Bar plots display the red blood cell aggregation index during model establishment; line plots present its variation over the drug administration period (n=6).

Changes in EG in rats during modelling and administration

During the modelling period, the EG progressively deteriorated. Compared with the Control group, EG thickness in the glomerulus was reduced, the podocyte cytoskeleton collapsed, and podocyte fusion was evident at 1 week after modelling. By 2 weeks, the glomerular EG had almost completely disappeared, podocytes were fully fused, and most had detached from the basement membrane. After 3 weeks of administration, no significant improvement in EG was observed in the Model group compared with the Control group. In contrast, the M+S group exhibited a marked restoration of EG structure, particularly at week 3 of administration, accompanied by partial recovery of podocyte morphology. Prednisone acetate treatment (M+Pa) did not produce a significant repair of EG; however, partial podocyte recovery was observed after 2 weeks of administration. Western blot analysis showed that, relative to the Control group, the expression levels of Glypican-1, CD44, and Syndecan-1 were decreased in the Model group. Compared with the Model group, the M+S group displayed significantly higher expression of CD44 and Glypican-1. Urinary analysis of HS and HA revealed that, compared with pre-modelling values, urinary HS increased after 1 week of modelling, and both HA and HS were elevated after 2 weeks. After 3 weeks of treatment, HS and HA levels remained significantly higher in the Model group than in the Control group ($P < 0.05$; $P < 0.01$). Compared with the Model group, M+S treatment significantly reduced urinary HS and HA, whereas M+Pa treatment produced no significant changes (Fig. 4).

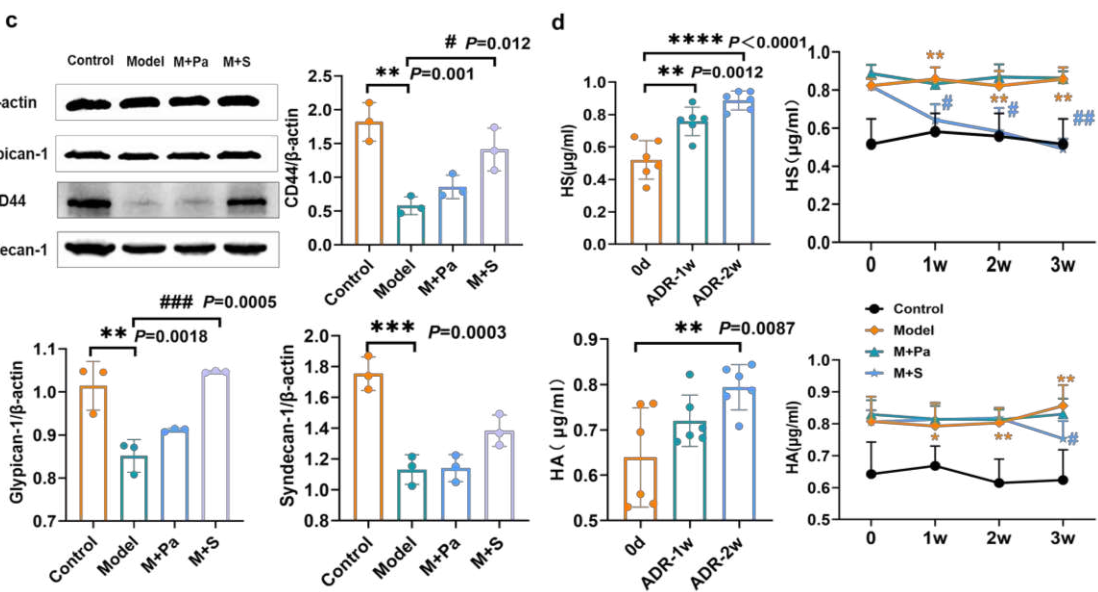
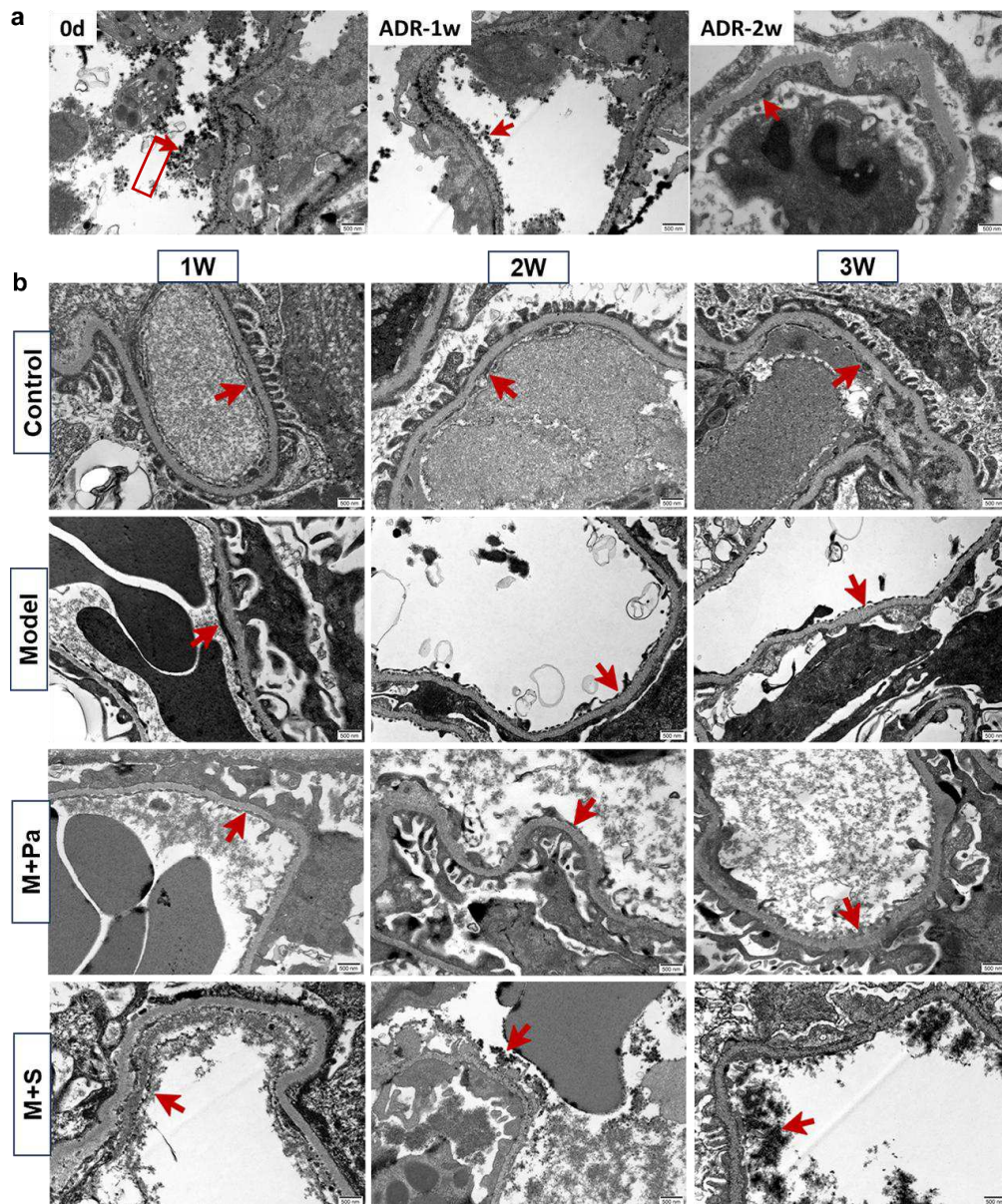


Fig.4. Alterations in the EG during model establishment and drug administration in rats. (a) TEM during model establishment. TEM showing progressive EG degradation in the glomerulus at baseline (0 d), 1 week, and 2 weeks after ADR injection; red boxes highlight the EG region (n=3). (b) TEM under different treatments. Representative TEM images of the glomerular EG from the Control, Model, M + Pa, M + S groups, dynamically observed after 1 week, 2 weeks, and 3 weeks of drug administration (n=3). (c) EG protein expression. Immunoblot analysis of core scaffold proteins (Syndecan-1, Glypican-1, and CD44) in glomerular lysates from each treatment group following drug administration (n=3). (d) EG component levels. Urinary HS and HA concentrations in the glomerular EG. Bar plots represent values during model establishment; line plots show temporal changes during drug administration (n=6).

Urinary albumin excretion and related pathological changes in rats during modelling and administration

Urinary albumin measurements at different time points showed that, compared with pre-modelling values, urinary protein levels were significantly increased at 1 week after modelling and continued to rise as modelling progressed. After drug administration, no significant decrease in urinary protein was observed in the Model group compared with the Control group. In contrast, both the M+Pa group and the M+S group exhibited significant reductions in urinary protein excretion at 2 weeks after administration. HE staining revealed normal morphology of the glomeruli and renal tubules in the Control group, with clear glomerular capillary structures, an intact glomerular basement membrane, evenly distributed mesangial matrix, and no evidence of edema or inflammatory cell infiltration in the renal interstitium. In the Model group, glomeruli were markedly enlarged, with some Bowman's capsules showing expansion, thickening of the capsule walls, and hyperplasia of mesangial cells and matrix. Substantial interstitial edema and focal inflammatory cell infiltration were observed. In prednisone acetate-treated and sulodexide-treated rats, kidney sections showed only mild congestion of glomerular capillaries, mild proliferation of mesangial cells, mild interstitial edema, and no obvious inflammatory cell infiltration. Immunohistochemical analysis demonstrated that, compared with the Control group,

VCAM-1 and ICAM-1 expression in renal glomerular tissue was increased in the Model group. Compared with the Model group, both the M+S and M+Pa groups exhibited significantly lower expression levels of VCAM-1 and ICAM-1 ($P < 0.05$; $P < 0.01$) (Fig. 5).

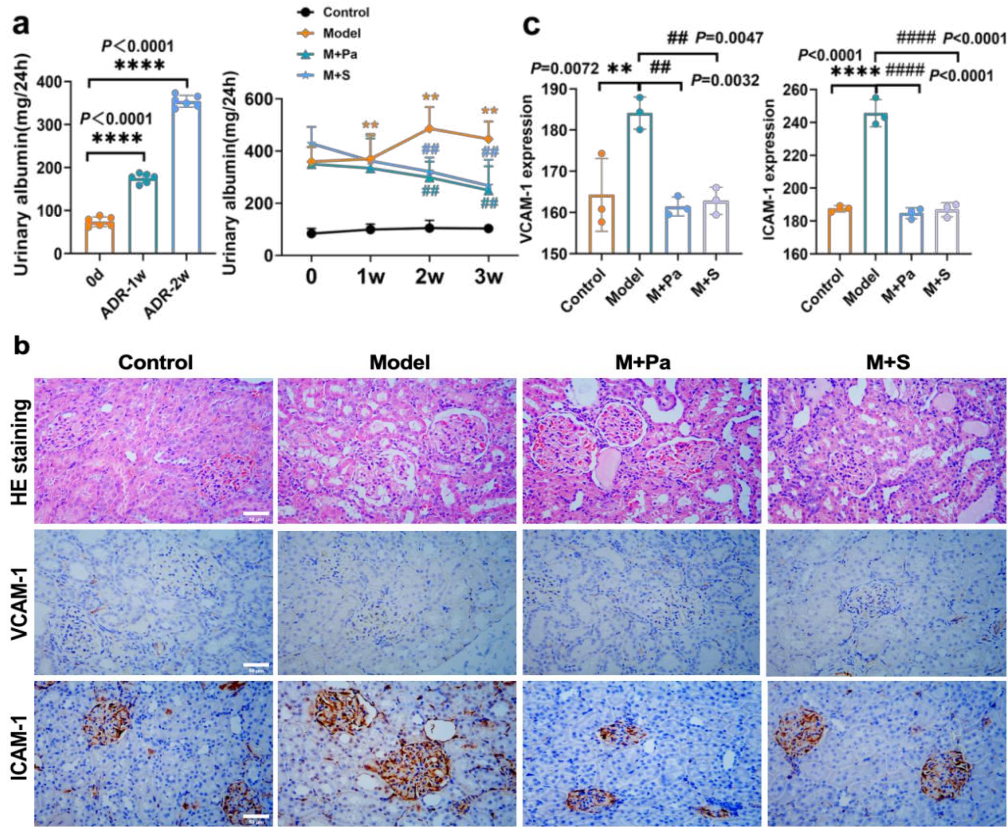


Fig.5. Urinary albumin excretion, glomerular histopathology, and adhesion molecule expression during model establishment and drug administration in rats. (a) Urinary albumin excretion. Bar plots represent values during model establishment, and line plots show temporal changes during drug administration ($n=6$). (b) Histopathology and immunohistochemistry. Representative images of rat glomeruli after drug administration, stained with HE and immunohistochemically labeled for VCAM-1 and ICAM-1 ($n=3$). (c) Quantitative adhesion-molecule expression. Bar plots show VCAM-1 and ICAM-1 expression levels in glomerular endothelial cells from each treatment group after drug administration.

Effects of different flow rates on cell and EG-related factors

In vitro experiments demonstrated that exposure of cells to a flow rate of $2 \mu\text{L}/\text{min}$ for 24 h resulted in good cell viability and the formation of relatively intact EG structures. Therefore, this flow condition was selected as the “smooth blood

circulation” state for establishing a physiological EG model, and subsequent pathological investigations were conducted on this basis. Under 2 $\mu\text{L}/\text{min}$, endothelial cells gradually elongated and aligned predominantly parallel to the direction of fluid flow, forming a relatively dense villous structure. As the flow rate decreased, cellular alignment became irregular, and cell morphology changed from spindle-shaped to oval-shaped. The density of villous structures progressively declined, and at 0 $\mu\text{L}/\text{min}$ (static culture), these structures were scarcely detectable. When the FSS was 5.185 dyn/cm^2 or 2.683 dyn/cm^2 , intracellular microfilaments were oriented along the long axis of the cells, stress fibers were well formed, and the expression levels of VCAM-1 and ICAM-1 were reduced. In contrast, at 0 dyn/cm^2 or 1.296 dyn/cm^2 , F-actin organization in HUVECs was disordered, microfilaments were thinner, stress fibers were absent, and VCAM-1 and ICAM-1 expression was increased (Fig. 6).

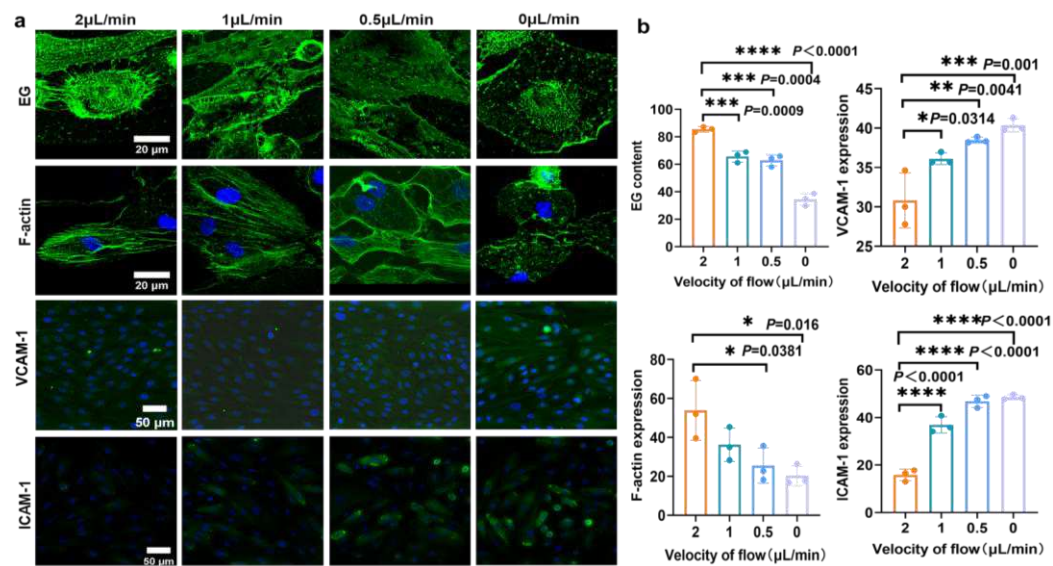


Fig.6. Representative images and quantitative analyses of EG morphology, cytoskeletal organization, and adhesion-molecule expression under different flow rates. Endothelial cells were subjected to flow rates of 2, 1, 0.5, and 0 $\mu\text{L}/\text{min}$, followed by immunofluorescence visualization of EG structure, F-actin cytoskeletal arrangement, and quantitative measurement of VCAM-1 and ICAM-1 expression levels (n=3).

Effect of EG on glomerular filtration barrier function

The results indicated that, following 24 h of circulation at a flow rate of 2 $\mu\text{L}/\text{min}$, the permeability of the GOAC progressively declined with increasing dextran

molecular weight. In contrast, reductions in flow rate were associated with a diminished EG and a corresponding gradual increase in GOAC permeability (Fig.7).

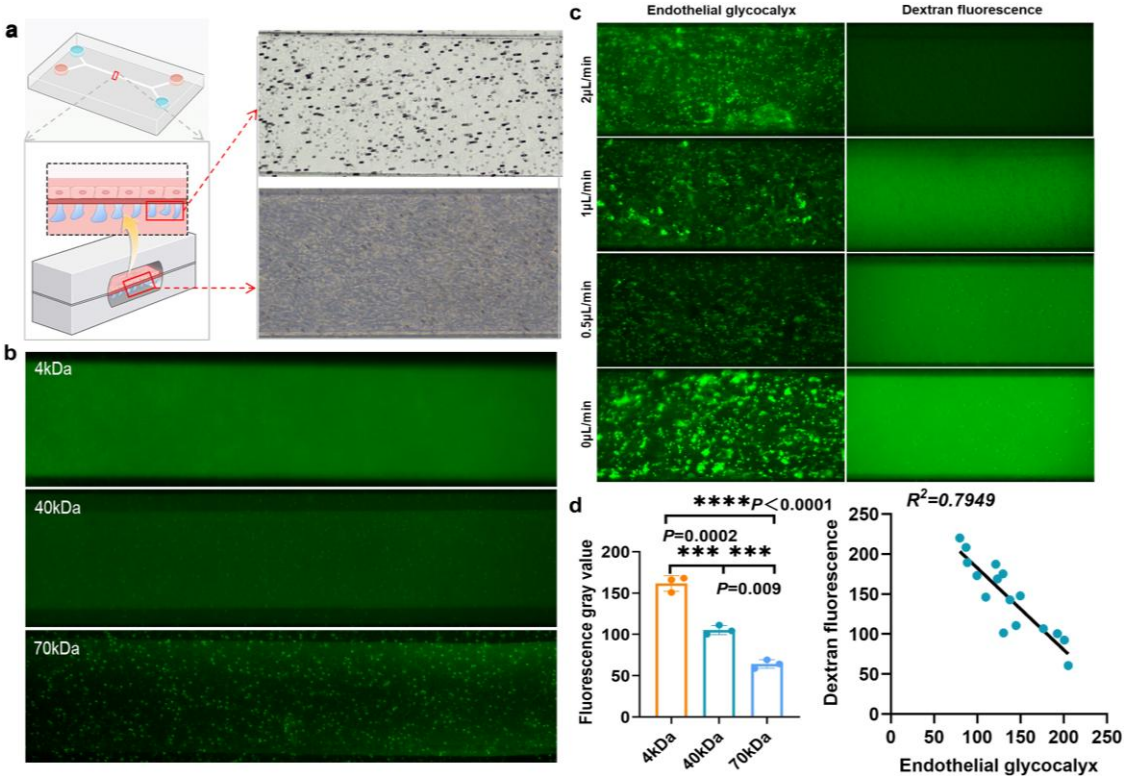


Fig.7. Structural characterization and barrier function assessment of the GOAC. (a) Device design and cell-seeding visualization. Schematic illustration and structural dissection of the GOAC, including vascular and urinary channels separated by a porous membrane, along with bright-field images of the GFB after seeding only HUVECs or co-seeding HUVECs with podocytes (n=3). (b) Selective permeability under physiological flow. Permeability of fluorescent dextran molecules with different molecular weights at a perfusion rate of 2 $\mu\text{L}/\text{min}$. (c) EG integrity and barrier function under varied flow. EG expression and permeability to 40 kDa dextran at different flow rates (n=4). (d) Relationship between EG expression and barrier function. Correlation analysis between EG expression levels and GOAC permeability.

Discussion

The present study demonstrates that rats with NS exhibit shortened PT and APTT, concomitant with elevated FBG levels. These findings align with previous reports describing dynamic fluctuations in coagulation parameters with disease activity. Mittal et al. reported APTT shortening during disease relapse, attributing this

phenomenon to urinary loss of albumin and anticoagulant factors, which subsequently triggers increased hepatic synthesis of procoagulant factors^[33]. Conversely, other studies, including a clinical investigation by Odimegwu et al., observed APTT prolongation in pediatric NS patients despite elevated fibrinogen levels during recurrence, while PT remained largely unaltered^[34-36]. These discrepancies may reflect variations in patient cohorts, disease stages, or methodological specifics. Physiologically, PT reflects the extrinsic coagulation pathway, whereas APTT primarily assesses the intrinsic pathway. Our results indicate concurrent activation of both coagulation cascades in NS rats. Previous studies have further established that a dysregulated balance between procoagulant and anticoagulant systems ultimately predisposes NS patients to both hemorrhagic and thrombotic complications^[37,38], supporting the presence of hemodynamic disturbances-namely a hyperviscous, hypercoagulable (“UBC”) state-in this condition.

In addition to coagulation and anticoagulation imbalance, NS rats in this study exhibited increased whole-blood viscosity and RBC aggregation indices. These hemorheological alterations may be linked to hyperlipidemia, hemoconcentration secondary to edema-induced hypovolemia, and increased plasma fibrinogen. Such changes further indicate abnormal blood flow in NS. Under physiological flow conditions, RBCs and leukocytes travel in the axial stream, platelets form a peripheral layer, and an outer cell-free plasma layer separates formed elements from the vessel wall, thereby preventing platelet-endothelium contact. However, when flow decelerates or vortices develop, platelets migrate into the plasma layer, increasing their likelihood of endothelial contact and adhesion.

EG, as the first barrier to blood flow, spatially segregates leukocytes, platelets, and albumin from direct endothelial contact, which is critical for preserving vascular integrity and normal hemodynamics^[39,40]. Beyond its barrier function, the EG acts as a key mechanosensor and responder to FSS^[41,42]. In vitro studies have shown that increasing FSS promotes EG reconstruction, with EG expression positively correlating with shear magnitude compared to static culture. Within a physiological range, higher flow velocities produce greater FSS and higher EG expression. In

hypercoagulable states, blood often exhibits “thick, viscous, sticky, and aggregated” characteristics. Flow deceleration and reduced FSS impair EG integrity. Notably, in patients with type 2 diabetes, endothelial cell abnormalities correlated more closely with urinary albumin excretion than podocyte injury, suggesting that endothelial damage may be a more critical determinant of glomerular injury than the traditionally emphasized podocyte injury^[43]. Ebefors et al. provided early evidence of cross-talk between activated podocytes and glomerular endothelium, reporting that EG degradation and proteinuria can precede podocyte foot process effacement^[44]. Multiple studies have established that glomerular endothelial cells and the EG form the initial barrier of the GFB, and their impairment constitutes an early step toward proteinuria in chronic kidney disease. In the present work, EG injury coincided with podocyte cytoskeletal collapse, but EG injury did not clearly precede podocyte damage, potentially owing to temporal limitations of our observation window, warranting further investigation.

Endothelial cells reside within a complex biochemical-biophysical microenvironment. While extracellular matrix components, growth factors, and hormones govern cellular behavior at the biochemical level, alterations in the mechanical microenvironment are detected by mechanosensory receptors, which subsequently regulate endothelial phenotype and function^[45-46]. Within the circulation, FSS plays an essential role in maintaining endothelial cell morphology and function^[47]. The cytoskeleton is central to endothelial mechanosensing and mechanotransduction. According to the “tensegrity” model, cytoskeletal components-microfilaments (notably F-actin), microtubules, and intermediate filaments-form an interconnected network, with F-actin being critical for cell shape and motility^[48,49]. Our results demonstrate that under conditions of an intact EG, actin filaments align with the cell's longitudinal axis and develop into organized stress fibers. EG damage results in thinner actin filaments and loss of stress fibers, indicating that EG-mediated FSS is vital for cytoskeletal homeostasis. Furthermore, vascular EG integrity critically influences endothelial function and modulates leukocyte-endothelium interactions. Importantly, we observed that decreased flow

rates were accompanied by upregulation of adhesion molecules VCAM-1 and ICAM-1. These molecules are mechanosensitive and can detect alterations in hemodynamic shear forces. Supporting this, a rat model of hypertension-induced cerebrovascular injury demonstrated that endothelial activation, increased VCAM-1 and ICAM-1 expression, and promoted neutrophil and monocyte adhesion and transmigration. The resulting endothelial injury and enhanced permeability contributed to cerebral edema. These findings collectively support a close association between VCAM-1/ICAM-1 upregulation and endothelial dysfunction.

Conclusion

In conclusion, our findings demonstrate that the EG is likely a central mediator in the pathophysiological process described as “UBC Results in WR.” EG injury in nephrotic syndrome may result from abnormal FSS secondary to a hypercoagulable state. Structural degradation of the EG was associated with upregulation of vascular endothelial adhesion molecules, endothelial dysfunction, and increased vascular permeability. These alterations may represent a critical mechanistic link between hemodynamic abnormalities and downstream renal injury. It should be noted that the EG comprises multiple molecular components, and the present study focused exclusively on the impact of blood flow dynamics on EG integrity and its related functional consequences. The biological roles of exfoliated EG components were not addressed and remain to be elucidated. Future studies will aim to characterize these components and clarify their contributions to vascular and glomerular pathology^[50].

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