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A cost-effective vessel-on-a-chip for high shear stress applications in vascular biology

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ABSTRACT

The vascular endothelium is constantly subjected to hemodynamic forces, including tangential shear stress, which are crucial for maintaining vascular homeostasis. Pathological shear stress levels, such as those observed in pulmonary arterial hypertension (PAH) or atherosclerosis, disrupt this balance, driving vascular remodeling and endothelial dysfunction. Current microfluidic platforms for studying these conditions are limited by high costs, excessive reagent requirements, and non-physiological channel geometries. Here we introduce a novel microfluidic chip system, a Nylon Vessel-on-a-Chip (NVoC) which represents a cost-effective and straightforward fabrication platform that eliminates the need for specialized equipment and enables a physiologically relevant round channel geometry. The NVoC was fabricated using Polydimethylsiloxane (PDMS) and nylon threads, with surface activation achieved through polydopamine and collagen-I coating, enabling robust endothelial cell (EC) attachment and long-term culture. Immortalized endothelial colony-forming cells (iECFCs) and human umbilical vein EC (HUVECs) were used to optimize and validate the platform, demonstrating its compatibility with high shear stress conditions (up to 90 dyne/cm^2) and various molecular biology techniques, including RT-qPCR, Western blotting, and immunofluorescent staining. With fabrication costs six times lower than commercial alternatives and overall experimental costs reduced threefold, the NVoC offers the ability to expose endothelial cells to physiological and pathological shear stress levels in a reproducible, accessible, and scalable manner. Its versatility and affordability make it a valuable tool for investigating shear stress-related mechanisms in microvascular diseases, particularly PAH, with potential applications in drug discovery and translational research.

1. Introduction

The vascular endothelium, which forms the inner lining of blood vessels, is directly exposed to blood flow, and is constantly subjected to hemodynamic forces, including tangential shear stress. This fluid shear stress plays a pivotal role in regulating endothelial cell (EC) function, influencing ECs ability to maintain vascular homeostasis in response to both chemical and mechanical stimuli (Hahn and Schwartz, 2009; Charbonier et al., 2019). However, pathological levels of shear stress may disrupt this balance, therefore contributing to the progression of various microvascular diseases. Conditions such as pulmonary arterial hypertension (PAH) are characterized by abnormally elevated shear stress due to vascular remodeling and increased pulmonary vascular resistance, leading to endothelial dysfunction and disease progression. Computational modeling studies consistently highlight the pivotal role of these hemodynamic changes in PAH, revealing that vascular remodeling and altered blood flow dynamics lead to abnormally high shear stress and mechanical strain in the pulmonary arteries, further exacerbating the disease (Dong et al., 2021; Yang et al., 2019; Bartolo et al., 2022). These findings underscore the necessity of in vitro systems capable of replicating such pathological shear stress conditions to better understand PAH pathophysiology and evaluate potential therapeutic

Abbreviations: BMP, Bone Morphogenetic Protein; EC, endothelial cell; iECFC, immortalized endothelial colony forming cells; NVoC, Nylon Vessel-on-a-Chip; PAH, pulmonary arterial hypertension; PDMS, polydimethylsiloxane; PD, polydopamine.

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strategies. Several commercially available systems, such as the ibidi μ-Slides, provide platforms for exposing ECs to shear stress and have greatly advanced the field of vascular research (Jatzlau et al., 2023). However, these systems often require significant volumes of medium and reagents, adding on the relatively high cost of the chips and associated perfusion systems, making them less accessible to resource-limited settings. Therefore, extensive research is performed on the fabrication of low-cost microfluidics for cell applications using the rapid prototyping approach (Kratz et al., 2019; Li et al., 2022). Though, up to now, none of them are capable to maintain the high shear stresses required to study PAH (Meng et al., 2022; Xie et al., 2024). Moreover, the rectangular geometry of the channels in many standard platforms limits their physiological relevance by failing to replicate the rounded lumen of native blood vessels. To address these challenges, the development of affordable, easy-to-use, and physiologically relevant microfluidic platforms is critical. By simplifying the cell culture workflow, such platforms could make shear stress experiments more accessible, encouraging a shift from static culture conditions to dynamic models as the new norm in endothelial cell research.

We designed a Nylon Vessel-on-a-Chip (NVoC) platform to fill this knowledge gap by combining cost-effective materials, such as polydimethylsiloxane (PDMS) and nylon threads, with a straightforward fabrication process that eliminates the need for specialized equipment. PDMS is widely regarded as a preferred material in academic research owing to its simple fabrication process and advantageous characteristics, such as optical transparency, gas permeability, low cost, nano-scale precisions and compatibility with biological systems (Halldorsson et al., 2015; Fiddes et al., 2010; McDonald and Whitesides, 2002). However, its inherently hydrophobic nature limits cell adhesion (Mata et al., 2005). To address this limitation, previous studies have developed effective surface activation techniques for PDMS, including polydopamine (PD), enabling long-term cell culture and expanding its applicability in biological research (Chuah et al., 2015; Kuddannaya et al., 2013; Trantidou et al., 2017). PD offers a robust and versatile surface modification strategy, forming conformal coatings on a wide range of substrates under mild, aqueous conditions. In addition, to improving surface hydrophilicity, it provides functional groups that enable the attachment of biomolecules, such as peptides or proteins, thereby enhancing compatibility with biological systems (Ryu et al., 2018). Additionally, reusable 3D-printed molds in our approach further enhances the system's practicality and sustainability. A key feature of the NVoC is the rounded channel geometry, which more closely mimics the structure of native vasculature compared to rectangular channels. The chip is designed to achieve a wide range of shear stress levels, from physiological to pathological, making it suitable for diverse research applications. Shear stress plays a central role in vascular biology, making the chip essential for disease modeling. Defined as the hydrodynamic force per unit area (dyne/cm^2 or Pa) exerted by blood flow on the vessel wall, shear stress varies widely, with physiological levels ranging from approximately 10 dyne/cm^2 in large arteries to 50 dyne/cm^2 in smaller arteries (Rodríguez and González, 2014; Givens and Tzima, 2016). Under pathological conditions, such as progressive PAH and congenital heart defects, shear stress can exceed 100 dyne/cm^2 in distal pulmonary arteries (100–500 μm) due to inward remodeling and luminal narrowing (Shinohara et al., 2024). This highlights the NVoC's potential for studying shear stress-related pathologies. Importantly, the NVoC delivers RNA and protein yields comparable to those of established platforms, addressing a common limitation of many customized microfluidic devices, which often struggle to generate sufficient material for downstream biochemistry and molecular biology techniques like qPCR and Western Blotting.

In this study, we describe the design, fabrication, and validation of our NVoC, demonstrating its ability to sustain EC cultures under both physiological and pathological shear stress conditions. To enhance cell attachment, we employed PD coating in combination with collagen-I, a strategy that improved cell adhesion, enabled long-term culture, and facilitated exposure to pathological high shear stress levels (Chuah et al., 2015). Furthermore, we developed a new immortalized endothelial colony-forming cell line (iECFCs), which are patient-derived yet offer the advantage of an unlimited cell source, making them ideal for reproducible and scalable experiments. As proof-of-principle, we demonstrated the chip's utility for molecular biology applications, including RT-qPCR, Western blotting, and immunofluorescent staining.

Mutations in the Bone Morphogenetic Protein Receptor Type 2 (BMPR2), leading to reduced BMPR-II expression levels and a pathological shift towards increased Transforming growth factor (TGF)-β signaling is the most common genetic defect in PAH (Lane et al., 2000). This imbalance drives vascular remodeling and endothelial dysfunction, hallmarks of PAH (Humbert et al., 2019). Therefore, we used this platform to specifically investigate signaling pathways involved in the development of PAH, such as the Bone morphogenetic protein (BMP) 9-phosphorylation of the downstream effector SMAD1 (pSMAD1) and induction of ID1, a BMP target gene (Miyazono et al., 2000; David et al., 2007).

Collectively, we show that our NVoC is an accessible, versatile, and cost-effective tool for studying shear stress-related effects in PAH, a central focus of our group's research. It provides an ideal platform to investigate the interplay between mechanical forces and BMP signaling pathways, offering valuable insights into endothelial dysfunction and potential therapeutic targets in PAH.

2. Materials and methods

2.1. Microfluidic chip fabrication

NVoC structures were designed using Autodesk Fusion360 (Fig. 1), and the molds were printed with a Formlabs FORM3b 3D printer using Biomed Clear resin (Formlabs, Berlin, Germany). The chips were fabricated by thoroughly mixing the PDMS base (silicone elastomer) with a curing agent at a ratio of 10:1, using the SYLGARD 184 Silicone Elastomer Kit. The components were manually mixed with a spatula for 1-2 min, ensuring a uniform consistency followed by degassing in a vacuum desiccator (#15544625, Fisher Scientific) for 45 min to eliminate air bubbles that were created during the mixing procedure. To ensure high transparency of the chip, a polymer film (90106 NB Arcare®, Adhesives Research) of low surface roughness was adhered to the inner side of the 3D printed mold. For channel formation, nylon threads (Glorex, Switzerland) with calibrated diameters of 0.35 mm, 0.4 mm, 0.5 mm, and 0.8 mm were threaded through pre-designed guide holes in the mold and tensioned using pipette tips to maintain a straight alignment (Fig. 2C). To define the inlet and outlet ports, nylon threads were threaded through short segments (5 mm) of PTFE tubing (2.0 mm outer diameter), which were then positioned and fixed at both ends of the mold to create well-aligned entry and exit points for the channels. The degassed PDMS mixture was carefully poured into the mold around the nylon threads, taking care to avoid bubble formation and cured in an incubator or oven at 60 °C for a minimum of 6 h, but typically over night to ensure full curing. After complete polymerization, the chip was carefully removed from the mold, using a spatula, a process facilitated by applying 70 % ethanol between the PDMS and the mold to reduce adhesion. Thread removal was conducted using one of two approaches: (1) application of a compressed air gun for 5–10 s at the thread entry site to reduce friction, followed by steady unidirectional pulling of the thread; or (2) allowing the chip to rest at room temperature for 24 h to promote natural shrinkage of the PDMS around the thread, enabling manual removal without air. To prevent residual PDMS from being dragged into the microchannels during removal, threads were pulled slowly with forceps and cleaned at both ends before final extraction. Once cleaned from PDMS, the thread was cut on one side, and a syringe fitted with a needle slightly larger than the thread diameter was used to flush the channel with 70 % ethanol. Ensuring that the needle encircled the thread allowed the ethanol to coat the surrounding surface,

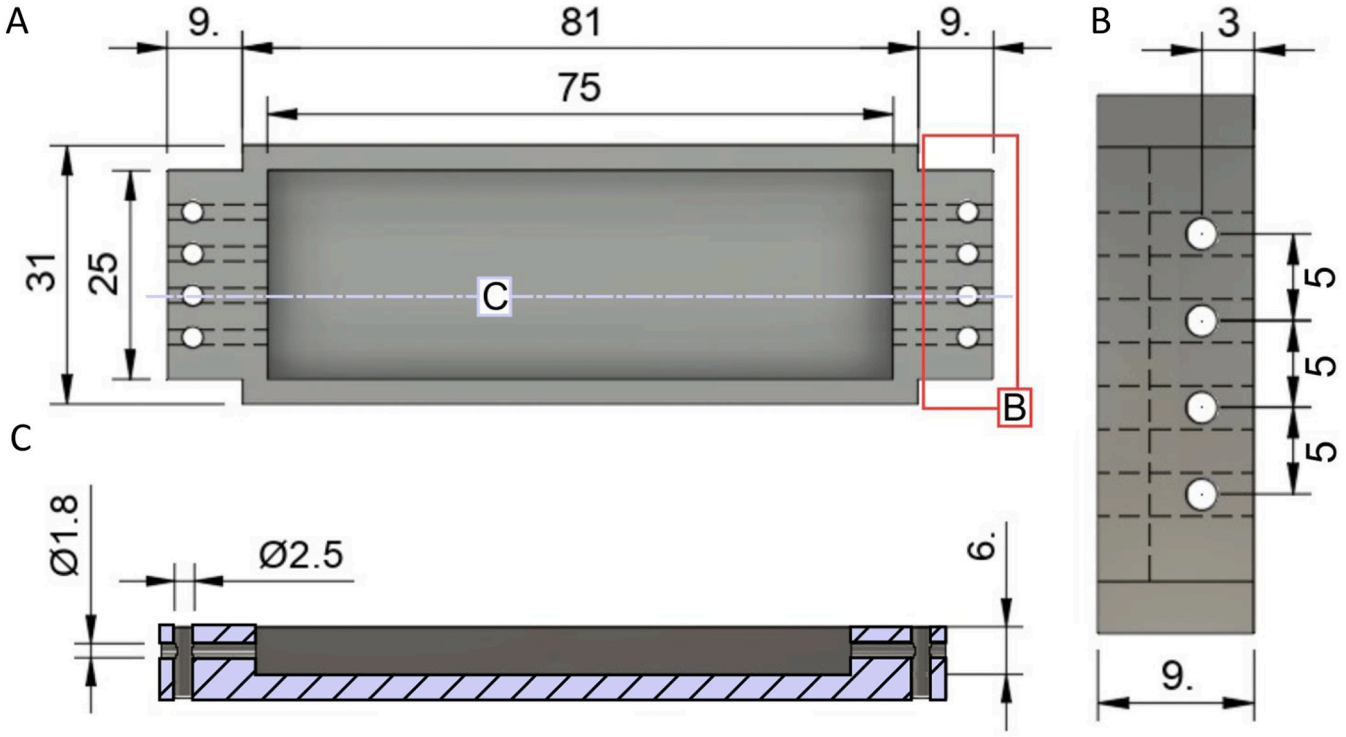


Fig. 1. Schematic representation of the mold features. (A) Top view, (B) zoom in and (C) side view of the mold used for NVoC fabrication. Dimensions are shown in mm. The holes for threading nylon threads have a diameter of 1.8 mm, and are 5 mm apart from each other, while larger holes (2.5 mm diameter) are included to secure the nylon threads using 10 µL pipette tips. The inner cavity of the mold, which forms the PDMS chip, has dimensions of 75 × 25 × 6 mm.

facilitating smooth and frictionless removal of the remaining nylon. This method proved particularly effective for minimizing mechanical stress during extraction. Overall, these procedures ensured the formation of homogeneous channels. Next, silicone tubing (1.0 mm inner diameter, 2.0 mm outer diameter) was attached to the inlets and outlets to either connect channels within a single chip or to aid the cell seeding. To verify channel integrity, all channels were first flushed with 70 % ethanol to remove any residual debris and ensure unobstructed flow. Visual inspection under a stereomicroscope was then performed using a colored solution to enhance contrast and clearly visualize the channel geometry. This allowed confirmation of continuous, debris-free lumens throughout the chip. For sterilization, the channels were flushed with 70 % ethanol, then dried over night at 60 °C until all ethanol had fully evaporated. Chips were exposed to UV light (365 nm) for 15 min prior to use.

2.2. Surface activation techniques

Surface activation techniques were evaluated to facilitate cell attachment to the PDMS surface of the NVoC. Sterilized chips were used for these experiments, with collagen coating alone (without prior surface activation) serving as the negative control. The first surface activation method involved O_2 plasma treatment, performed using an Atto plasma chamber (Diener electronic GmbH & Co. KG, Ebhausen, Germany). Chips were subjected to varying treatment durations, including 1 min as a single exposure, 4 cycles of 20 s each, and 6 cycles of 30 s each at 40 kHz and 200 W. As a second surface activation approach, PD treatment was employed. For this, 2 mg/mL dopamine hydrochloride was freshly dissolved in 10 mM Tris-HCl buffer (pH 8.5) prior to each treatment. The solution was filtered to ensure sterility, and the channels were treated with the PD solution for 1 h at room temperature under sterile conditions. Following treatment, the channels were thoroughly flushed with sterile Milli-Q water, with three washes performed, each involving a 10-min incubation period. The channels were dried by aspirating residual water, and the chips were either used immediately or stored for use the following day.

Following either surface activation technique, the channels were coated with 100 µg/mL collagen type I (C3867, Sigma-Aldrich) for 1 h at 37 °C. The collagen solution was subsequently replaced by phosphate-buffered saline (PBS) and then by the appropriate culture medium in preparation for cell seeding.

2.3. Cell culture

Circulating endothelial colony-forming cells (ECFCs) were isolated during routine blood tests from healthy donors as described before (Sánchez-Duffhues et al., 2019). Immortalization was outsourced to InSCREENeX GmbH. In short, for lentiviral transduction of the gene library, the cryopreserved cells were thawed and plated in culture medium on a 12-well plate. After reaching 80 % confluency, the primary cells were transduced with lentiviral vectors encoding a gene library composed of 33 different expansion genes, as described previously (Lipps et al., 2018). Lentiviral transduction was performed overnight at a multiplicity of infection (MoI) of 5 in the presence of polybrene (8 µg/mL; Sigma-Aldrich). On the following day, virus-containing media was aspirated, and the cells were cultivated for additional 5 days before selection with medium containing 0.2 mg/mL G418 (Thermo Fisher, Germany). The transduced cells were selected for 14 days with medium renewal every 4 days, and colonies of proliferating cells became visible. Resulting colonies were pooled and further expanded without any selection pressure and used for further characterization. Primary ECFCs were cultured in EGM-2 medium (#CC-3162, Lonza), on 1 % (*w/v*) gelatine-coated cultureware. Immortalized ECFCs (iECFCs) were cultured on 1 % (*w/v*) collagen-coated standard culture ware (Corning) using MCDB 131 medium (Gibco™, Thermo Fisher Scientific) supplemented with 100 U/mL Pen/Strep, 10 % fetal bovine serum (FBS), 1 µg/mL hydrocortisone, 2 mM L-glutamine, and 10 ng/mL vascular endothelial growth factor. For validation experiments, human umbilical vein endothelial cells (HUVECs) were cultured in EGM-2 medium (#CC-

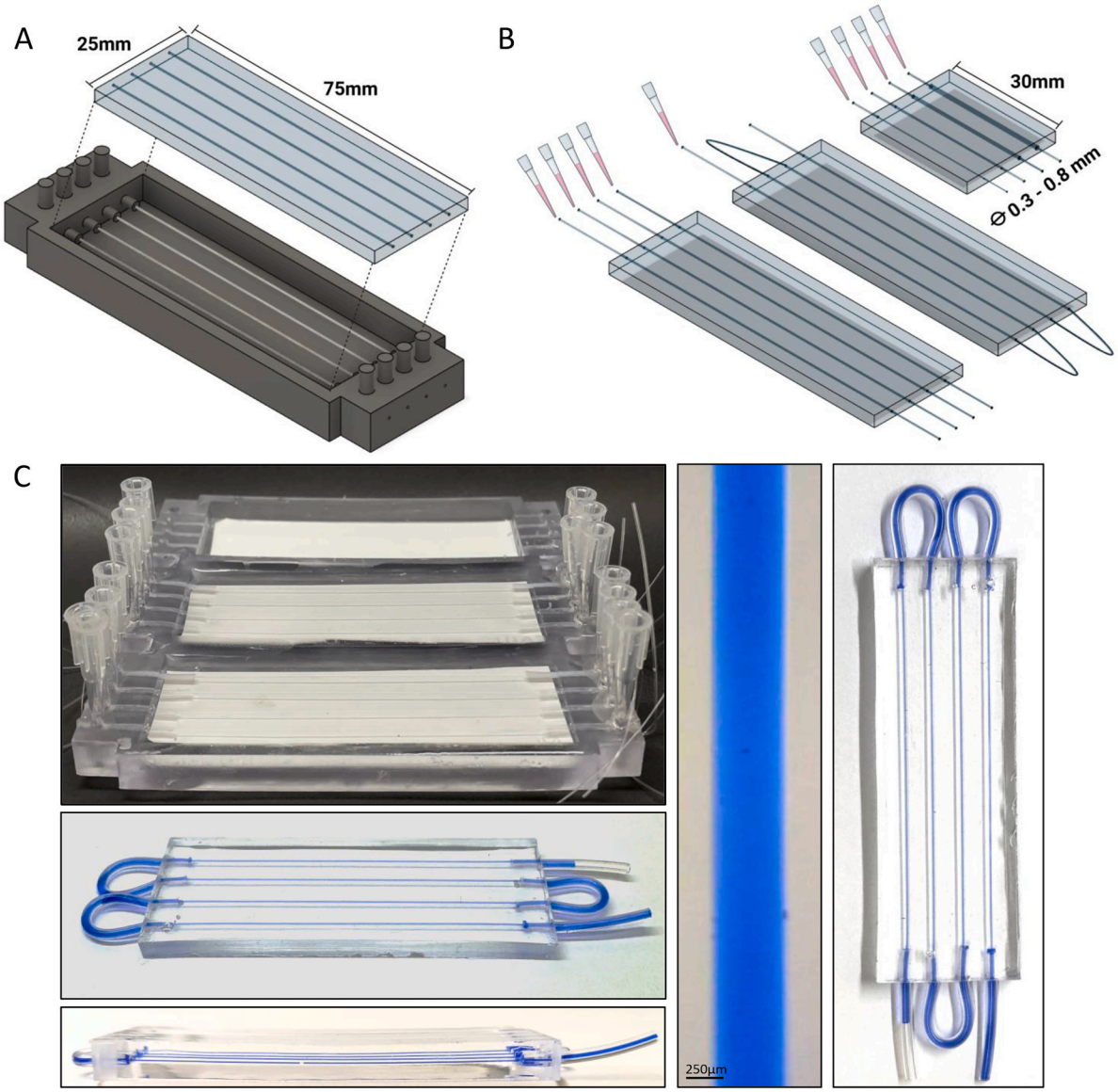


Fig. 2. Schematic representation of the NVoC casting and design versatility. (A) The casted PDMS chip features standard objective slide dimensions (25 × 75 mm) and includes four parallel round channels. (B) The versatile design allows rapid customization of chip size, channel dimensions, and the use of the four channels either independently or in combination. (C) Examples of the chip casting process and finished PDMS chips.

3162, Lonza), on 0.1 % (w/v) gelatine-coated cultureware. All cell lines were maintained in a humidified atmosphere at 37 °C with 5 % CO_2, and cell cultures were routinely tested to confirm the absence of mycoplasma contamination.

2.4. Shear stress experiments

Cells were seeded into surface-activated and collagen-coated NVoCs. Two seeding strategies were evaluated: (1) the chips were inverted every 30 min over a 4-h period, or (2) cells were allowed to attach for 2 h, after which a second seeding step was performed following chip inversion, with an additional 2-h attachment period. On the same day as seeding, the cells were subjected to perfusion with cell culture medium using either a peristaltic pump (CON-SP-KIT-001, BMT) or a pressure-based pump system (ibidi pump system quad, #10906, ibidi GmbH). For static conditions, PDMS-coated 12-well plates were prepared following the same sterilization, surface activation, and collagen-coating procedures as described for the microfluidic chips (refer to 2.1.). For initial experiments, cells were exposed to shear stress levels of 20 dyne/cm^2 for 1 to 7 days, beginning with a ramp phase of 3 h to ensure gradual adaptation to the flow conditions. As an additional control, cells were seeded into µ-Slide I $^{0.6}$ Luer (ibidi, #80186), µ-Slide I $^{0.2}$ Luer (ibidi, # 80166) or µ-Slide 4 well (ibidi, #80426) for static conditions. These slides served as widely used and validated control microfluidic devices. After shear stress exposure (2.5 dyne/cm^2, 20 dyne/cm^2 or 90 dyne/cm^2) or maintenance under static conditions, cells were starved for 6 h in MCDB 131 basal medium supplemented with 0.1 % FBS and 100 U/mL penicillin/streptomycin and stimulated with BMP9 (1 ng/mL) (R&D, #3209-BP-010/CF) or left untreated for molecular biology assays.

2.5. Reverse transcription-quantitative PCR (RT-qPCR)

For RT-qPCR analysis, 2 × 10^5 cells were seeded into either NVoC or PDMS-coated 12-well plates in MCDB 131 medium and perfused at 20 dyne/cm^2 or maintained under static conditions for 36 h. Starved cells were then stimulated with 1 ng/mL BMP9, or a ligand buffer for 1 h. To isolate RNA, cells were first washed with PBS and then trypsinized within the channels or wells for 4 min at 37 °C. The resulting cell

suspension was collected in medium containing 10 % FBS to neutralize the trypsin. This step was repeated as needed to recover all cells from the channels. The cell suspension was then centrifuged at 5000 ×*g* for 1 min, and the resulting pellet was washed once with PBS. Cells were lysed using the lysis buffer provided in the RNA Miniprep System (Promega, #Z6012), and RNA extraction was performed according to the manufacturer's protocol. RNA yield was quantified using a NanoDrop spectrometer (Thermo Fisher, #61531). A total of 300 ng RNA was reverse-transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, #K1632). RT-qPCR was conducted using the GoTaq® SYBR Green Supermix (Promega, #A6001) on a CFX384 Connect Real-Time PCR System (Bio-Rad) in accordance with the manufacturer's instructions. Ct values for genes of interest were normalised to those of housekeeping genes GAPDH and ARP using the ΔΔCt method. Primer sequences used are provided in the supplemental material (Table S1).

2.6. Western blotting

For Western blot analysis, 2×10^5 cells were seeded into the NVoC with MCDB 131 medium. After 36 h of exposure to shear stress (20 dyne/cm^2), cells were starved for 6 h in MCDB 131 basal medium containing 0.1 % FBS and 100 U/mL penicillin/streptomycin. Starved cells were then stimulated with 1 ng/mL BMP9 or a ligand buffer for 45 min. Cells were subsequently harvested as described in Section 2.5. Proteins were extracted by lysing cells in radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors (Roche, #11836145001) and phosphatase inhibitors, including 10 mM sodium fluoride (Sigma, #7681-49-4) and 400 μM sodium orthovanadate (Sigma, #S6508). Protein concentrations were quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher, #23235), following the manufacturer's instructions. Equal amounts of protein (20 μg) were denatured in Laemmli buffer and separated on 10 % polyacrylamide gels using SDS-PAGE. Proteins were transferred onto 0.2 μm PVDF membranes (Biorad, #170457) using the trans-blot turbo transfer system (Biorad, #1704150) and blocked for 1 h at room temperature with 10 % non-fat dry milk in tris-buffered saline containing Tween 20 (TBST). Membranes were incubated overnight at 4 °C with primary antibodies diluted in TBST. The following antibodies were used: phospho-SMAD1/5/9 (Cell Signaling, #13820), total SMAD1 (Cell Signaling, #6944), and vinculin (Sigma-Aldrich, #V9131) as a loading control. Working concentrations are listed in the supplemental material (Table S2). After washing with TBST, membranes were incubated for 1 h at room temperature with secondary antibodies (1:5000 dilution) in TBST with 10 % milk. Secondary antibodies included anti-mouse HRP (Promega, #W4021) and anti-rabbit HRP (Invitrogen, #31458). Protein signals were developed using the Western Bright Quantum HRP substrate (Advansta, #K-12042-D20) and visualized using the ChemiDoc Imaging System (Bio-Rad, Hercules, CA).

2.7. Immunofluorescent staining

To visualize cellular structures within the NVoC, cells were fixed in 4 % paraformaldehyde for 20 min at room temperature after a gentle wash with PBS. Fixation was followed by quenching with 2 mg/mL glycine and permeabilization using 0.2 % Triton X-100 for 10 min. Non-specific binding sites were blocked by incubating the cells with 5 % bovine serum albumin (BSA; Sigma-Aldrich, #A-6003) in PBS. Primary antibodies targeting CD31 (R&D Systems, #AF3628) or VE-Cadherin (Cell Signaling, #2158) were diluted in 1 % BSA in PBS and applied to the samples overnight at 4 °C. Following incubation, cells were washed extensively with a solution containing 0.5 % BSA and 0.05 % Tween-20 in PBS to remove unbound antibodies. Secondary detection was performed in the dark at room temperature for 1 h using Alexa Fluor-conjugated secondary antibodies and AlexaFluorPlus647 Phalloidin (Invitrogen, #A30107) to visualize actin filaments. Working concentrations are listed in the supplemental material (Table S2). Subsequent to the secondary labelling, the nuclei were stained with DAPI (Thermo Fisher) for 10 min, followed by three washes with PBS. After staining, sodium azide was added to the channels, and the chips were stored in a humidified environment at 4 °C to preserve fluorescence. Imaging was conducted using a SP8 confocal microscope (Leica) or the confocal Dragonfly 500 (Andor) to capture details of the cellular morphology. *Z*-stack imaging and IMARIS Analysis Software was used for the 3D reconstruction of the channel geometry.

2.8. Statistical analysis

Data analysis and visualization were conducted using GraphPad Prism version 9 (GraphPad Software). Experimental data were analyzed through one-way or two-way ANOVA, followed by Tukey's post-hoc test to account for multiple comparisons, with specifics provided in the figure legends. Statistical significance was defined as a p-value ≤ 0.05.

3. Results

3.1. Development of a PDMS-based microvascular chip with straight and round channels

The primary objective of this microvascular device design was to achieve high shear stress levels in laminar flow. To realize this, we developed a PDMS-based chip containing straight and round channels. A custom 3D-printed mold was designed to incorporate nylon threads and a mechanism to maintain their tension. The chip format was based on the dimensions of a standard object slide (25 mm × 75 mm), allowing the inclusion of four parallel straight channels (Figs. 1 and 2A). This versatile design supports rapid customization of chip size, channel count, and channel diameter, depending on experimental requirements (Fig. 2B). By using nylon threads of varying diameters (0.3 mm to 0.8 mm), the channel dimensions could be precisely adjusted. Tensioning of the threads was achieved using 10 μL pipette tips, positioned within designated holes in the mold. Upon removal of the nylon threads, clean, straight channels were embedded within the PDMS chip (Fig. 2C). This streamlined approach enables the production of microvascular devices optimized for high shear stress applications, while maintaining adaptability to a range of experimental setups. The chip's varying diameter and length provide a cell cultivation area per channel of 0.7 to 1.9 cm^2, comparable to the cultivation area of a 48-well and 24-well plate, respectively.

3.2. Characterization of immortalized endothelial colony forming cells

Circulating ECFCs were isolated from healthy donors and subjected to immortalization by Inscreenex GmbH to generate immortalized ECFCs (iECFCs), as described previously (Fig. 3A) (Lipps et al., 2018). ECFCs, being primary cells, are a valuable resource for research as they can also be derived from patient samples and recapitulate pathogenic mechanisms of diseases (Sánchez-Duffhues et al., 2019; van de Pol et al., 2019). However, their use is limited by the technically challenging, labor-intensive culture requirements, including daily medium changes with endothelial growth medium (EGM-2) to maintain viability, homogeneity and functionality, adding complexity and cost to their handling. Therefore, the immortalization of ECFCs offers several advantages. First, it provides an unlimited supply of cells for the evaluation of novel technologies in biological applications. Additionally, it allows the use of a more cost-effective endothelial culture medium (MCDB 131) and simplified cell culture practices. Importantly, iECFCs exhibited no observable morphological differences compared to primary ECFCs under both static and flow conditions. Neither primary nor immortalized ECFCs showed alignment to the flow direction when exposed to a shear stress of 20 dyne/cm^2 (Fig. 3B). In standard culture, primary ECFCs are maintained on gelatin-coated (1 %) substrates. However, collagen is the

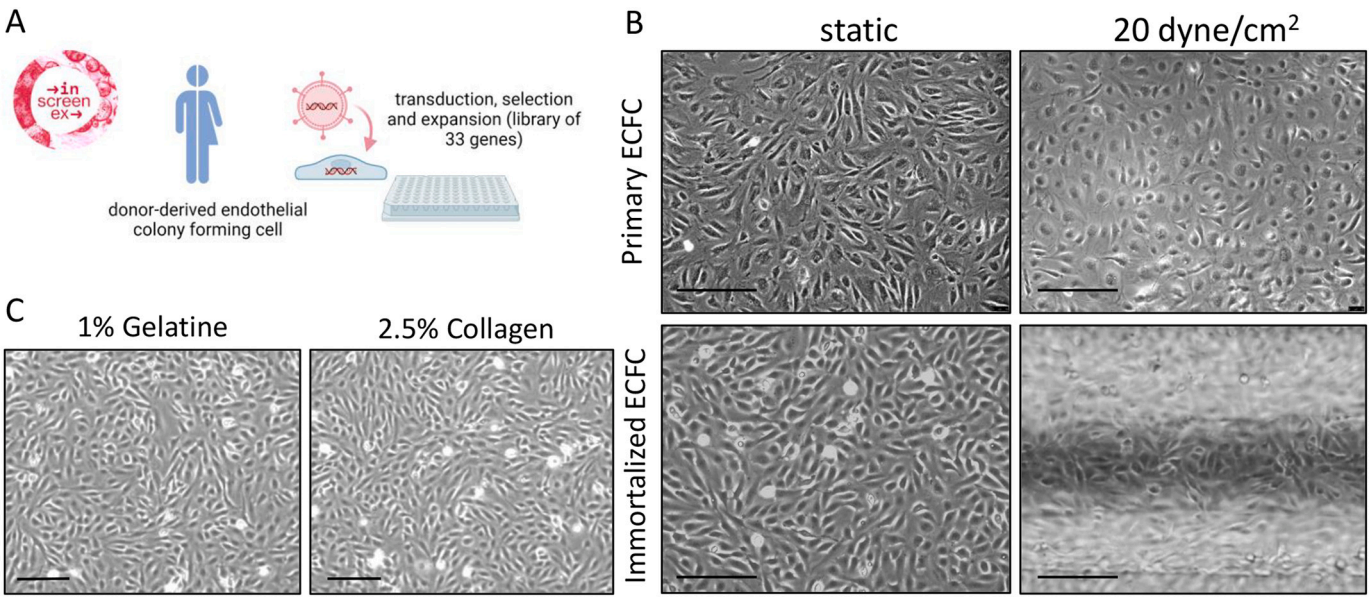


Fig. 3. Characterization of immortalized endothelial colony-forming cells (iECFCs). (A) Schematic representation of the iECFC immortalization process performed by Inscreenex. (B, C) Morphological comparison of immortalized ECFCs and primary ECFCs when cultured in different endothelial cell media (B) or when cultured on 1 % gelatin versus 1 % collagen extracellular matrix (C). The scale bar represents 200 μm.

preferred extracellular matrix (ECM) for microfluidic applications. To assess the impact of ECM on cell morphology, we cultured iECFCs on a 2.5 % collagen matrix. No significant morphological changes were observed when transitioning from gelatin to collagen-coated surfaces (Fig. 3C). Overall, immortalized ECFCs retained key characteristics of primary ECFCs and standard culture conditions were optimized to include MCDB 131 medium and collagen as the ECM substrate, enabling cost-effective and reproducible experimentation.

3.3. Surface activation strategies of PDMS for cell attachment

Among widely used methods to enhance cell compatibility of PDMS are oxygen plasma treatment and extracellular matrix protein coating. Despite their effectiveness, these methods often suffer from short-lived effects due to the hydrophobic recovery of the PDMS surface (Akther et al., 2020). An alternative approach involves the use of bio-inspired PD, which reduces surface hydrophobicity, minimize in vivo toxicity of implanted biomaterials, and strongly bind substrates via covalent and non-covalent interactions (Chuah et al., 2015; Hong et al., 2011; Tsai et al., 2011; Dabaghi et al., 2021). Here, we tested three surface activation strategies to optimize cell attachment within the microchannels of the PDMS chips. In the first approach, the PDMS surfaces were coated with collagen type I prior to cell seeding. The second strategy involved oxygen plasma treatment (6 × 30 s at 40 kHz and 200 W) followed by collagen coating to render the surface more hydrophilic. In the third method, PD coating was applied up to 24 h before collagen coating. Neither collagen absorption alone nor oxygen plasma treatment with subsequent collagen coating provided sufficient enhancement of cell attachment to the PDMS surface after 2 h (Fig. 4A). In contrast, PD coating followed by collagen absorption resulted in robust cell attachment at both 2 and 24 h post-seeding (Fig. 4A, B) and was thus selected as the standard method to use in subsequent cell experiments. To optimize seeding density, we evaluated different cell concentrations and their attachment at 0 and 2 h post-seeding. A density of 2×10^5 cells per channel yielded a confluent monolayer after 2 h (Fig. 4B) and was suitable for short-term shear stress experiments (up to 48 h). This seeding density proved effective across various channel sizes (Fig. 4C). A single seeding step was used to evaluate surface attachment and for seeding optimizations. In summary, the combination of PD and collagen-I coating emerged as the optimal surface activation strategy for PDMS microchannels, ensuring effective cell attachment. A seeding density of 2×10^5 cells per channel consistently resulted in confluent channels, irrespective of channel dimensions, making this method suitable for diverse experimental setups.

3.4. Seeding strategies optimization for full endothelial coverage

The mathematical formula for calculating wall shear stress (τ) in a cylindrical channel is displayed in Fig. 5A, where μ is the fluid viscosity, Q is the volumetric flow rate, and R is the channel radius. This relationship illustrates how shear stress is influenced by channel dimensions and flow rates. To generate shear stress within the NVoC, a peristaltic pump with a maximum speed of 40 rpm was employed, producing a maximum flow rate of 800 μL/min. Flow rates per rpm were provided by the company and confirmed by manually measuring the weight of the medium, supplemented with 10 % FBS over a specific time interval (considered density 1000KG/m^3). Shear stress levels were calculated using the above formula, including the different channel diameters: channels with a diameter of 0.8 mm yielded a maximum shear stress of 2.03 dyne/cm^2, while smaller diameters of 0.5 mm, 0.4 mm, and 0.3 mm produced maximum shear stress of 8.3, 16.2, and 38.41 dyne/cm^2, respectively (Fig. 5B). These values were calculated using a fluid density of 1000 kg/m^3 and viscosity of 0.00072 Pa·s, representing cell culture medium supplemented with 10 % FBS. In addition, computational fluid dynamics (CFD) simulation was performed to validate our theoretical shear stress estimations within the microfluidic channel. The simulation confirms the calculated shear stress and gives additional insight on the shear stress and velocity distributions for each microchannel geometry and experimental flow rates used in our study. The 3D channel geometries, resulting heatmaps and line graphs for velocity and shear stress distribution across the channel cross-section, as well as convergence plots and mesh geometries, are provided in Supplementary Fig. S1 and Supplementary Table S3. To evaluate potential deformation of the PDMS channel under high shear stress, we performed live imaging with a colored solution, demonstrating that even the smallest channel geometry (0.35 mm) exhibited no visible deformation during perfusion at the highest flow rates (Supplementary Video 3). This supports the validity of the CFD simulation, as significant deformation would alter the channel geometry and thus affect the calculated shear stress values. To test whether complete endothelialization of the round channels could be

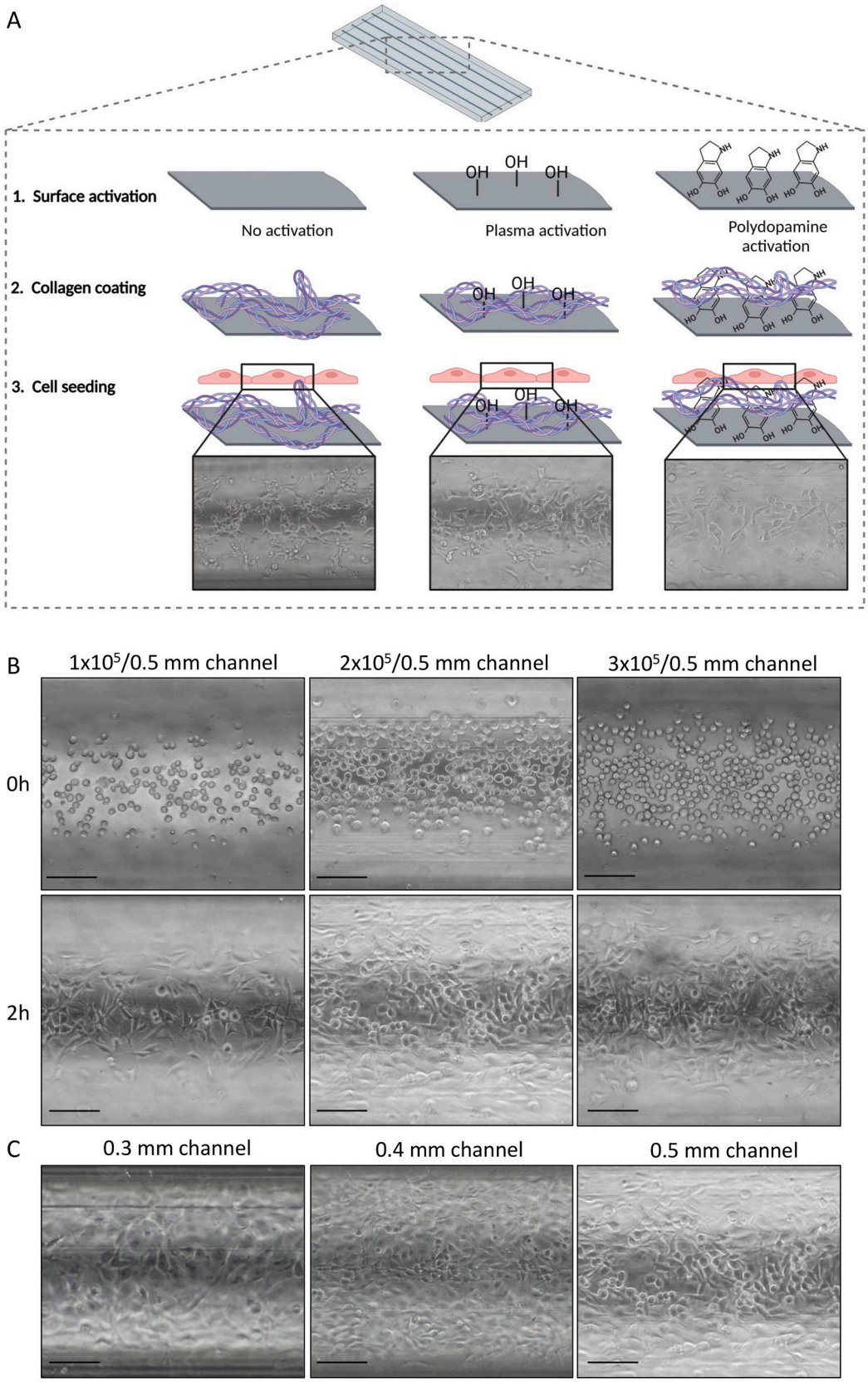


Fig. 4. Surface activation techniques and optimization of iECFC seeding in PDMS channels. (A) PDMS channels were subjected to one of three surface activation techniques: no activation, oxygen plasma activation, or PD coating, followed by collagen-I coating prior to cell seeding. Cell attachment was assessed 2 h post-seeding, before the start of perfusion experiments. (B) iECFCs were seeded at densities of 1×10^5, 2×10^5, or 3×10^5 cells per channel, and seeding density was evaluated at the time of seeding (0 h) and 2 h post-seeding. (C) A density of 2×10^5 cells per channel was tested across channels of different diameters (0.3 mm, 0.4 mm, and 0.5 mm) to evaluate its suitability for various channel sizes. The scale bar represents 200 μm.

achieved, a multi-step workflow was implemented. Following chip casting, sterilization, and surface activation with PD and collagen-I, cells were seeded at a density of 2×10^5 cells per channel. The chips were inverted every 30 min over a 4-hour period to promote uniform cell attachment (Fig. 5C). However, microscopy analysis revealed incomplete coverage of endothelial cells (ECs) across the channel surface. *Z*-stack imaging confirmed that this initial seeding strategy did not reliably achieve full endothelialization. To address this limitation, the seeding protocol was modified. After an initial 2-hour attachment period, the chips were inverted, and a second seeding step was performed. This dual-seeding approach resulted in full coverage of the round microchannels by ECs, as shown by *Z*-stack imaging. The imaging displayed a hollow lumen with confluent endothelial layers across two planes, confirming complete endothelialization (Fig. 5E, Supplemental Videos 1 and 2). This revised seeding protocol ensures reproducible and consistent coverage of EC, critical for generating physiologically relevant conditions in microfluidic experiments.

3.5. Validation to use the NVoC for biological applications

To evaluate the utility of the NVoC for diverse biological applications, we tested its compatibility with a pressure-based perfusion system (ibidi GmbH). This system employs air pressure, controlled via dedicated software, to regulate flow. Flow rates for different channel sizes were determined following the manufacturer's guidelines. Briefly, the chip was connected to the perfusion system using blunt-end needles, and the desired pressure was applied. The time t (in seconds) required to deliver 2 mL of medium was measured, with a minimum of five replicates taken to calculate the mean. Flow rate (ml/min) was then computed using the following formula:

$$\Phi\left[\frac{\mathrm{ml}}{\mathrm{min}}\right] = 2[ml]*60\frac{\left[\frac{s}{min}\right]}{t[s]}$$

Based on these flow rates, shear stress levels were calculated as described before, with a maximum shear stress of 90 dyne/cm^2 achieved for a 0.35 mm channel (Fig. 6A). Next, we explored the chip's application in molecular biology by assessing its suitability for qPCR and Western Blot analyses. One of the primary objectives in studying cellular responses to shear stress is comparing downstream signaling pathways under dynamic and static conditions. To address this, we tested whether ECs remained attached to the PDMS surface within the microfluidic channels in the absence of perfusion. However, significant cell death was observed after one day of culture under static conditions, whereas cells exposed to low shear stress (2.5 dyne/cm^2) exhibited robust survival (Fig. 6B). This observation suggests that the NVoC is not optimal for long-term static control experiments. Therefore, we assessed the use of standard cell culture well plates as a static control. Since differences in surface activation and coating could impact cell attachment, morphology, and downstream signaling, the well plates were coated with PDMS and subjected to the same preparation steps as the NVoC, including sterilization and surface activation with PD and collagen-I (Fig. 6C). This approach significantly improved and stabilized EC attachment for extended periods, rendering these plates suitable for use as static controls (Fig. 6D).

Furthermore we compared the long-term performance of the NVoC to the widely used commercially available ibidi $^{0.6}$ Luer channel chip. The EC monolayer remained intact after seven days of exposure to high shear stress levels (20 dyne/cm^2) in both systems (Fig. 6D). Collectively, these findings validate the NVoC's potential for biological applications, particularly under dynamic shear stress conditions, and establish PDMS-coated well plates as a viable static control platform. Next, we assessed the suitability of the NVoC for mRNA and protein analyses, focusing on its compatibility with techniques such as qPCR and Western Blot. Confluent EC layers were harvested from the NVoC channels and compared to those retrieved from the ibidi $^{0.6}$ Luer chip. Both systems yielded comparably high RNA and protein concentrations, sufficient to analyze multiple target genes or proteins in parallel (Fig. 6E and G). To demonstrate the chip's utility, we performed a proof-of-concept analysis by examining key signaling components of the BMP pathway, specifically *ID1* mRNA levels and pSMAD1 protein levels, in response to a BMP ligand highly potent in EC, such as BMP9 (Fig. 6F and H). These downstream targets were chosen due to our group's interest in TGF-β/BMP-related pathologies, including PAH. In addition, we tested whether staining of cells within the NVoC was feasible using conventional immunofluorescent staining protocols. To optimize staining conditions, we adapted the size of the mold to reduce the channel length to 30 mm (from the original 75 mm), facilitating the antibody to reach the interior of the microchannels. Imaging of stained structures, including nuclei (DAPI), EC (VE-Cadherin), and actin filaments (Phalloidin), was successfully achieved, as demonstrated in Fig. 6I. In summary, these findings confirm the NVoC's applicability for advanced molecular biological studies under dynamic shear stress conditions. Furthermore, the establishment of PDMS-coated well plates as a reliable static control system reinforces the versatility of the platform for a wide range of experimental applications.

3.6. Simulating high shear stress conditions to model pathological microenvironments

Computational modeling has demonstrated that abnormally elevated levels of shear stress are present in the distal pulmonary circulation of patients with idiopathic pulmonary arterial hypertension (PAH). These elevated shear stress levels arise due to inward vascular remodeling, which increases pulmonary vascular resistance (PVR) (Dong et al., 2021; Yang et al., 2019). Developing a reliable model to replicate these high-shear environments is critical for studying disease pathophysiology and testing potential therapies. To investigate the shear stress tolerance of iECFCs, we utilized our NVoC under maximal flow conditions achievable with 0.4 mm and 0.35 mm channels. These conditions generated shear stresses of 61 and 90 dyne/cm^2, respectively. Remarkably, the iECFCs maintained viability and integrity under these extreme shear stresses for at least 72 h (Fig. 7A). A ramp phase of 5 h was implemented to gradually increase the shear stress to the target levels, while allowing the iECFCs to adapt. By 24 h, the cells exhibited alignment along the direction of flow at both shear stress rates. In summary, these findings demonstrate the robustness of our NVoC as a platform to expose ECs to very high shear stress levels. This capability is essential for replicating pathological environments associated with microvascular diseases, offering new opportunities to explore disease mechanisms and potential therapeutic interventions in vitro.

3.7. Flow-induced alignment of HUVECs under shear stress

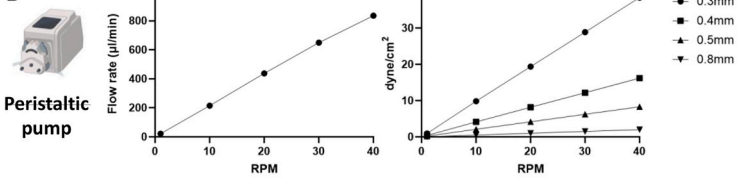
Human umbilical vein endothelial cells (HUVECs) were used to investigate whether the lack of flow alignment observed in both primary and immortalized ECFCs at shear stress levels of 20 dyne/cm^2 was attributable to the cell type or the NVoC design. HUVECs were cultured under static conditions or exposed to shear stress levels of 5 and 20 dyne/cm^2 for 72 h. Under static conditions, the cells displayed a randomly oriented morphology. However, when exposed to shear stress, HUVECs demonstrated a clear alignment in the direction of the flow. Alignment was evident after 72 h at both shear stress levels (Fig. 8A). These findings confirm that the NVoC supports the culture of EC types that respond to shear stress with morphological alignment, indicating that the lack of flow alignment in iECFCs is likely cell-type specific rather than a limitation of the chip design. To further assess the functional shear responsiveness of EC within the NVoC, we analyzed the expression of the shear-responsive gene *eNOS* in both HUVECs and iECFCs cultured under static, low (2.5 dyne/cm^2), and high (90 dyne/cm^2) shear stress conditions. Cells were exposed to flow for 48 h in either the NVoC (red bars) or the commercially available ibidi $^{0.6}$ Luer for 2.5

A

$$\tau = \frac{4\mu Q}{\pi R^3}$$

τ = shear stress (dyne/cm^2)
μ = viscosity
Q =flow rate (cm^3/s)
R = radius

B



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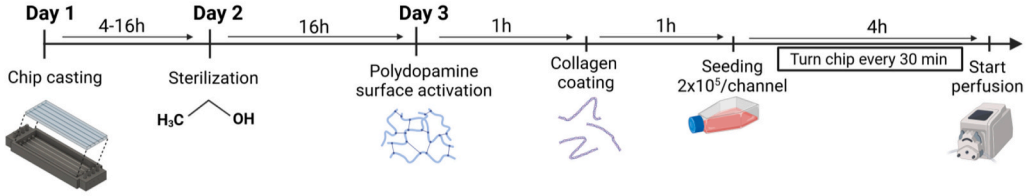

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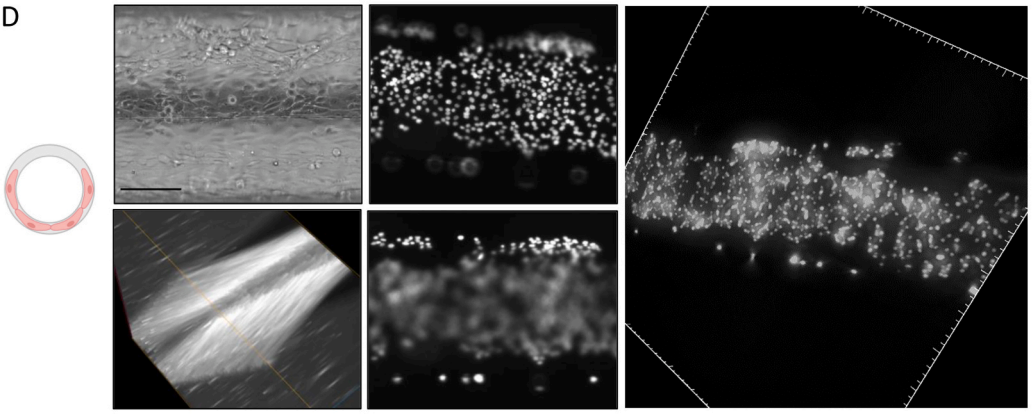

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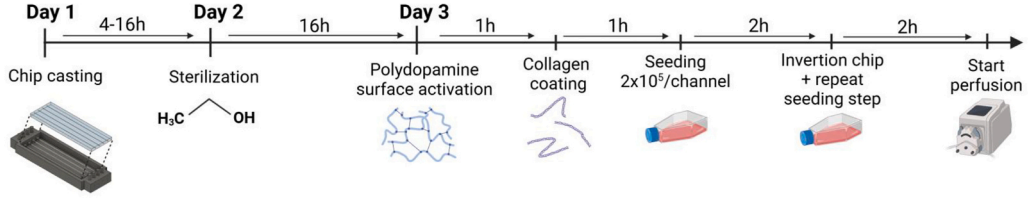

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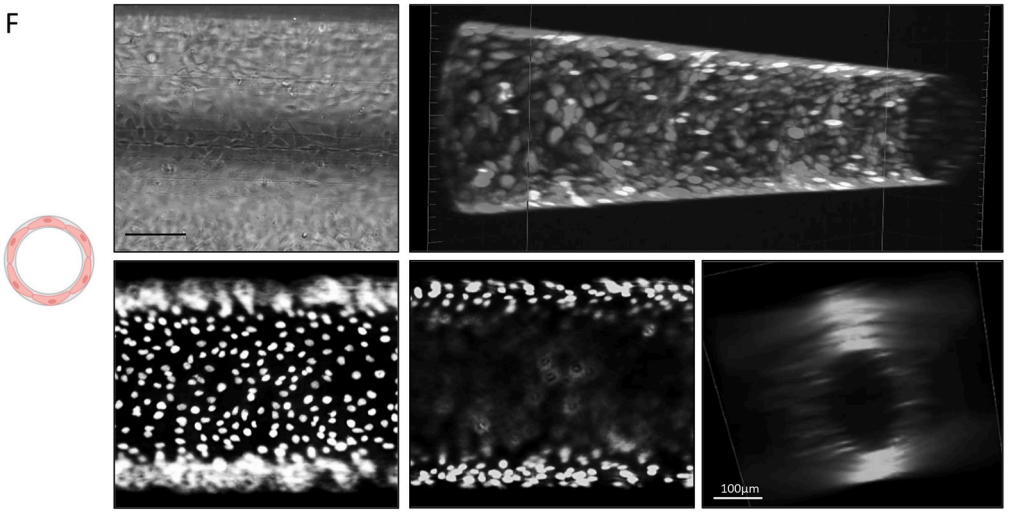

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Fig. 5. Optimizing seeding strategies for full endothelial coverage of the NVoC. (A) Formula for wall shear stress (τ) in a cylindrical channel. (B) Calibration of flow rate using a peristaltic pump with a maximum capacity of 40 rounds per minute (rpm), showing calculated shear stress levels for different channel sizes (0.3 mm, 0.4 mm, 0.5 mm, and 0.8 mm) based on a fluid density of 1000 kg/m^3 and viscosity of 0.00072 Pa·s. (C) Schematic representation of the initial seeding workflow, where chips were inverted every 30 min for 4 h to achieve complete endothelialization. (D) Microscopic evaluation using *Z*-stack imaging revealed incomplete endothelial coverage with this approach. (E) Schematic representation of the adjusted workflow, where cells were allowed to attach for 2 h before the chip was inverted, and the seeding step repeated. (F) Z-stack imaging confirmed full endothelialization of the channels using the adjusted workflow, showing a continuous endothelial lining forming a round vessel with a hollow lumen. The scale bar represents 200 μm.

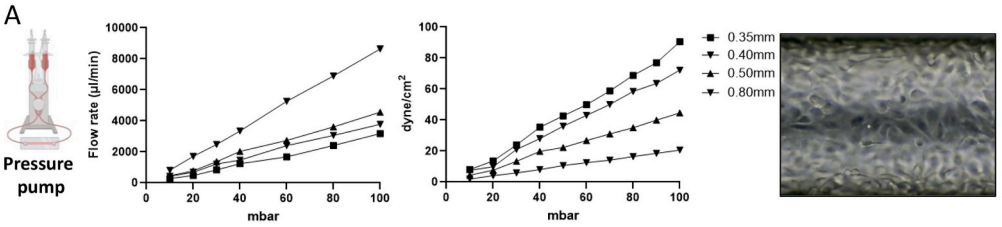
dyne/cm^2 or ibidi $^{0.2}$ Luer for 90 dyne/cm^2 (grey bars). As shown in Fig. 8B, *eNOS* expression was significantly upregulated under high shear stress in both cell types and in both systems, whereas low shear stress did not induce a comparable increase. These findings confirm the NVoC's capacity to elicit robust, physiologically relevant transcriptional responses. Brightfield images further demonstrate that both cell types remained viable under all conditions; however, only HUVECs showed clear morphological alignment in response to high shear, suggesting a cell-type-specific response. To investigate whether differences in cell junction integrity may underlie this observation, we performed VE-Cadherin staining in HUVECs after exposure to flow. As shown in Fig. 8C, VE-Cadherin localized to cell–cell junctions, consistent with EC monolayer integrity and alignment under flow conditions. This provides evidence that the lack of flow alignment in ECFCs is a cell type-specific behavior rather than a limitation of the chip design. Importantly, this result underscores the versatility of the microfluidic chip for studying diverse EC responses under physiological and pathological flow conditions.

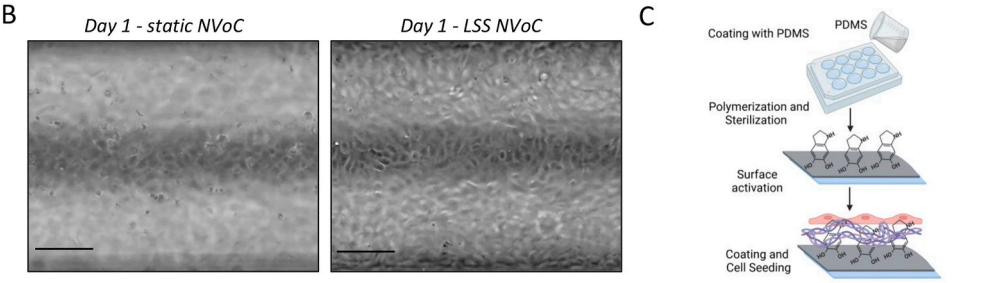
4. Discussion

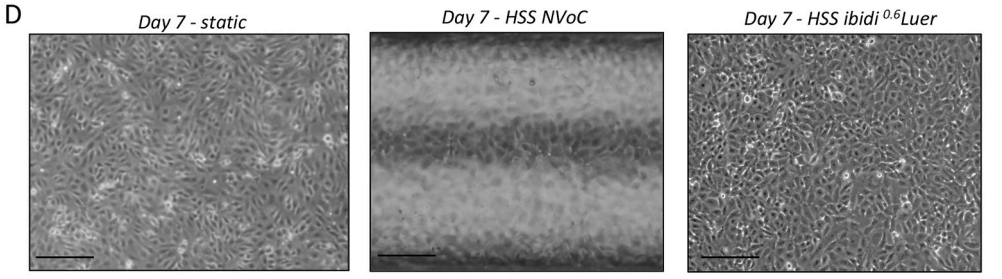
In this study, we have developed and characterized a novel PDMS-based vessel-on-a-chip (NVoC) that combines a cost-effective with a user-friendly design, capable of replicating physiologically relevant shear stress levels within a rounded geometry. By utilizing readily available materials and 3D-printed molds, this system offers an accessible alternative for laboratories specializing in vascular biology without a deep engineering background. Unlike many microfluidic platforms that require expertise in bioengineering and complex fabrication techniques, the NVoC can be manufactured and utilized in standard biological laboratories with minimal technical training. The ability to fabricate chips in-house without specialized equipment, alongside its affordability and adaptability, highlights the practicality of this approach for widespread use. The NVoC enables the generation of shear stress levels ranging from physiological to pathological, making it suitable to resemble for a broad spectrum of physiological environments within vascular biology. Its accessibility, scalability, and adaptability, enabled by reusable 3D-printed molds and reduced resource requirements, position it as a viable alternative to commercially available systems, particularly for resource-constrained laboratories. The reusable molds circumvent the need for continuous access to a 3D printer, ensuring consistent quality over time. Furthermore, pre-fabricated chips can be prepared in advance and stored for long-term use, offering flexibility in experimental workflows. While polydopamine (PD) has been widely employed to enhance cell adhesion in microfluidic devices due to its biocompatibility and ease of application, its long-term impact on cellular behavior remains an area of ongoing study. Previous work has shown that PD-coated PDMS surfaces significantly improve the adhesion and proliferation of HUVECs, highlighting its suitability for applications involving EC culture under dynamic condition (Yang et al., 2022). PD coatings alter ECM protein adsorption and integrin expression, including activation of focal adhesion kinase (FAK), suggesting that downstream pathways such as PI3K/Akt or MAPK may also be modulated, depending on the cellular context and ECM environment (Deng et al., 2021). In our high shear stress (61-90 dyn/cm^2) system, we neither observed adverse effects on morphology or viability after 3 days, nor did we observe visual degradation of the coating over typical culture periods. Future work could investigate the durability of PD under long-lasting flow in complex media, or explore surface pre-treatment and crosslinking strategies to enhance coating longevity. Our findings further demonstrate that both HUVECs and iECFCs exhibit a comparable transcriptional response to shear stress within the NVoC system, as evidenced by the upregulation of *eNOS* mRNA under high shear stress, consistent with results obtained using the commercially available ibidi Luer chip. However, notable differences in cellular behavior were observed between the two cell types. While HUVECs displayed clear VE-Cadherin localization at cell–cell junctions and aligned robustly in the direction of flow, iECFCs lacked junctional VE-Cadherin staining, despite comparable eNOS induction. This absence may explain their reduced alignment under shear conditions. Additionally, the proliferative nature of iECFCs appears to interfere with sustained alignment, as shown in Fig. 7 where a slight alignment is observed after 24 h of high shear stress but diminishes at 48 and 72 h. These findings suggest that the observed differences in alignment are likely attributable to inherent EC-type characteristics rather than the chip design. The chip's four parallel channels allow for the simultaneous testing of multiple experimental conditions, minimizing the risk of confounding variables and enhancing reproducibility. Combined with its capacity to achieve a wide range of shear stress levels, these features underline the system's potential for conducting high-quality, reliable vascular research. A key advantage of the NVoC is its significantly lower cost compared to commercially available microfluidic chips. A single ibidi Luer chip, including the endothelial medium required for the perfusion system and recombinant proteins for cell stimulation, costs approximately 30€ per experiment, with the chip alone priced at 16€ with one perfusable channel. In contrast, our NVoC enables experiments to be conducted for an average cost of 10€, with chip production costs of 2.50€ with 4 perfusable channels. This represents a more than sixfold reduction in chip fabrication costs and an overall threefold reduction in experimental expenses. The substantial cost savings derive from the NVoC's reduced medium consumption, lower recombinant protein requirements, and the use of widely available, inexpensive materials. Furthermore, unlike many custom vessel-on-a-chip platforms that rely on costly fabrication techniques such as soft lithography, high-resolution 3D printing, or micro-milling, our system eliminates the need for specialized equipment, further improving its accessibility.

The NVoC supports diverse molecular cell biology applications, including RT-qPCR, Western blotting, and immunofluorescent staining, enabling comprehensive analysis of EC responses to shear stress. Its compatibility with various assays makes it particularly well-suited for studying, for example BMP signaling pathways in PAH or evaluating responses of drugs targeting endothelial dysfunction (Jatzlau et al., 2023; Ntekoumes and Gerecht, 2022; Becher et al., 2024).

While the NVoC is optimized for dynamic flow applications, its suitability for long-term static culture is limited. In the absence of perfusion, significant EC death was observed within 24 h, highlighting the system's incompatibility with extended static conditions. To address this limitation, we developed an alternative approach using standard tissue culture well plates coated with PDMS and treated identically to the chip surface, including sterilization and functionalization with PD and collagen-I. This strategy significantly enhanced cell viability and attachment over extended periods. Moreover, qPCR analysis confirmed that gene expression in our static control setup was comparable to ibidi well plates (Fig. 8), which also use a surface-modified treatment (ibiTreat) similar in function to our custom coating. Looking ahead, the modular design of the NVoC offers promising opportunities for







E

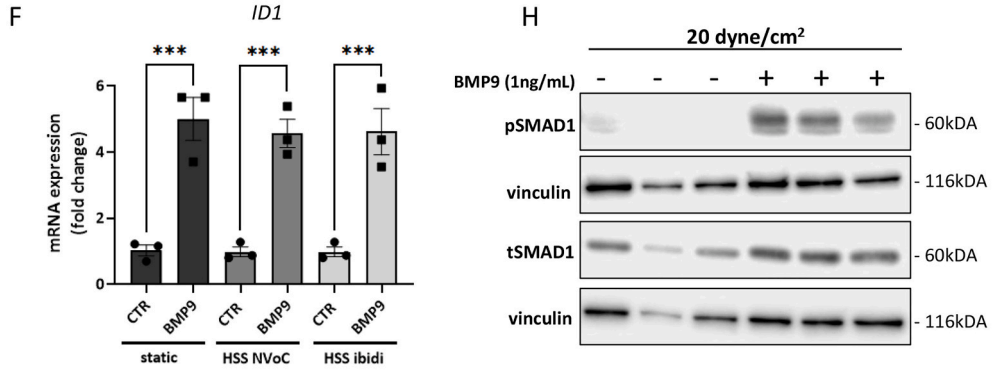
RNA yield

Model	Min. (µg)	x̄ (µg)	Max. (µg)
NVoC	0.64	1.4	2
Ibidi (*0.6Luer*)	0.85	1.8	2.3

G

Protein yield

Model	Min. (µg)	x̄ (µg)	Max. (µg)
NVoC	42.49	47.13	58.79
Ibidi (*0.6Luer*)	29.39	48.27	59.44



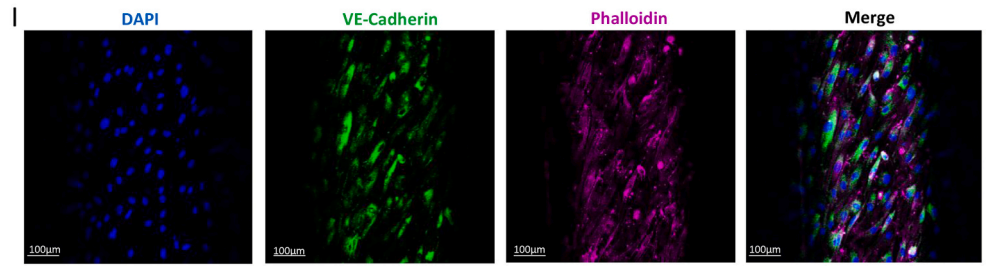


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Fig. 6. Validation of the NVoC for biological applications. (A) Calibration of flow rate using the ibidi pressure-based pump system for various channel sizes (0.35 mm, 0.4 mm, 0.5 mm, and 0.8 mm), with corresponding shear stress levels calculated. (B) Brightfield images comparing cell viability of iECFCs cultured in microfluidic channels under static (no perfusion) and low shear stress (LSS, 2.5 dyne/cm^2) conditions. (C) Schematic representation of 12-well plates coated with PDMS to simulate static conditions. The PDMS-coated plates were subjected to surface activation, collagen-I coating, and iECFC seeding to replicate the preparation steps used for the NVoC. (D) Brightfield images of iECFCs after 7 days of culture under static (PDMS surface) and high shear stress (HSS, 20 dyne/cm^2) conditions, comparing the NVoC and the ibidi $^{0.6}$ Luer chip. The scale bar represents 200 μm (E) Comparison of RNA yields (average, minimum, and maximum in μg) obtained from iECFCs cultured in NVoC versus ibidi Luer chips, based on at least five measurements. (F) Gene expression analysis of *ID1* in iECFCs under static or HSS (20 dyne/cm^2) conditions after 36 h of culture (NVoC vs. ibidi $^{0.6}$ Luer chip), with stimulation by BMP9 (1 ng/mL) or unstimulated controls. (G) Protein yield comparison (average, minimum, and maximum in μg) from NVoC and ibidi $^{0.6}$ Luer chips, based on at least five measurements. (H) Western blot analysis of phospho-SMAD1 (pSMAD1), total SMAD1 (tSMAD1), and vinculin (loading control) in iECFC samples cultured under HSS conditions (20 dyne/cm^2) in NVoC chips for 36 h, with BMP9 (1 ng/mL) stimulation for 45 min or unstimulated controls. (I) Immunofluorescent staining of iECFCs exposed to HSS (20 dyne/cm^2) for 48 h, showing endothelial marker VE-Cadherin, actin filaments (Phalloidin), and nuclei (DAPI). Statistical differences were tested using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons; $^{***}p < 0.001$. Graphs are displayed as mean + SEM.

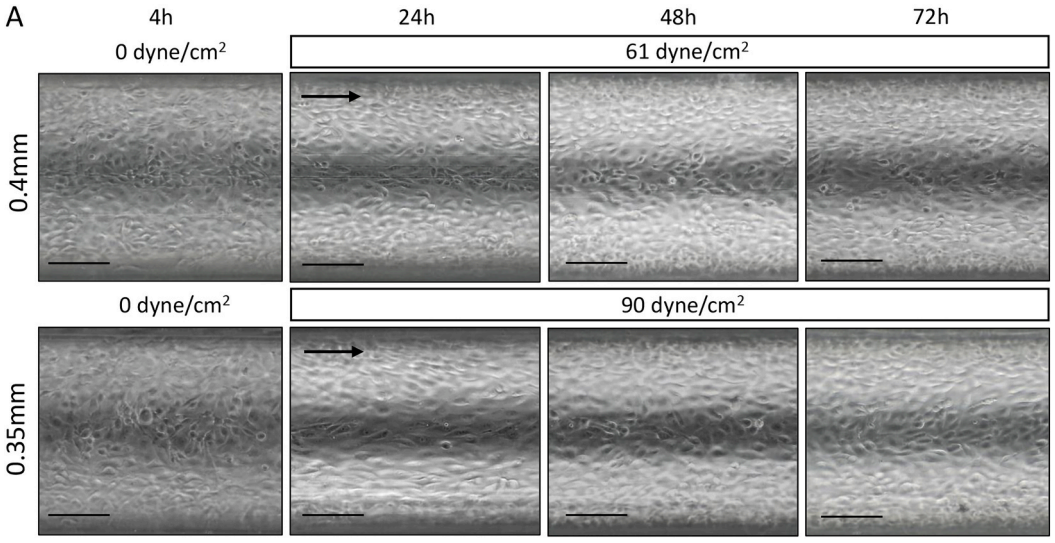

Fig. 7. Simulating high shear stress conditions to model pathological microenvironments. (A) iECFCs were cultured in the NVoC with channel diameters of 0.35 mm and 0.4 mm for 72 h, exposed to the maximum pressure achievable using the ibidi pump system, resulting in shear stress levels of 61 dyne/cm^2 and 90 dyne/cm^2, respectively. The arrow indicates the direction of the flow and the scale bar represents 200 μm.

incorporating additional vascular cell types to better mimic complex microenvironments. For example, previous studies have demonstrated the successful use of direct 3D co-culture systems by mixing EC with pericytes in defined ratios (van der Meer et al., 2013). The flexibility of the NVoC in channel geometry and seeding protocols allows for similar multi-step cell seeding strategies, facilitating the development of more physiologically relevant vascular models. In addition, the NVoC holds significant potential for investigating other diseases where high wall shear stress is implicated, such as intracranial aneurysms and atherosclerosis, but where its precise role in disease pathophysiology remains to be fully elucidated (Dolan et al., 2013).

In summary, the NVoC represents a robust and versatile platform for vascular research, offering a cost-effective and physiologically relevant alternative to existing microfluidic systems. Future studies integrating additional vascular cell types and more complex models are anticipated to further expand its utility for translational research and disease modeling.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mvr.2025.104814.

Translational impact statement

This study introduces a cost-effective vessel-on-a-chip system that mimics physiological and pathological shear stress conditions observed in vascular diseases, like pulmonary arterial hypertension. By enabling compatibility with molecular biology techniques, this platform offers a versatile tool for studying endothelial dysfunction, exploring disease mechanisms, and testing potential therapeutic interventions. Its affordability and ease of fabrication make it accessible to laboratories worldwide, promoting broader research into microvascular diseases and translational applications in vascular biology.

CRediT authorship contribution statement

Clarissa Becher: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martin Frauenlob:** Writing – review & editing, Supervision, Methodology. **Florian Selinger:** Writing – review & editing, Methodology. **Peter Ertl:** Writing – review & editing. **Marie-José Goumans:** Writing – review & editing, Resources, Funding acquisition. **Gonzalo Sanchez-Duffhues:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Ethical approval statement

This study was conducted in accordance with the Medical Ethical Committee from the Leiden University Medical Center (METC LUMC) and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All participants provided informed consent prior to their participation in the study.

Funding

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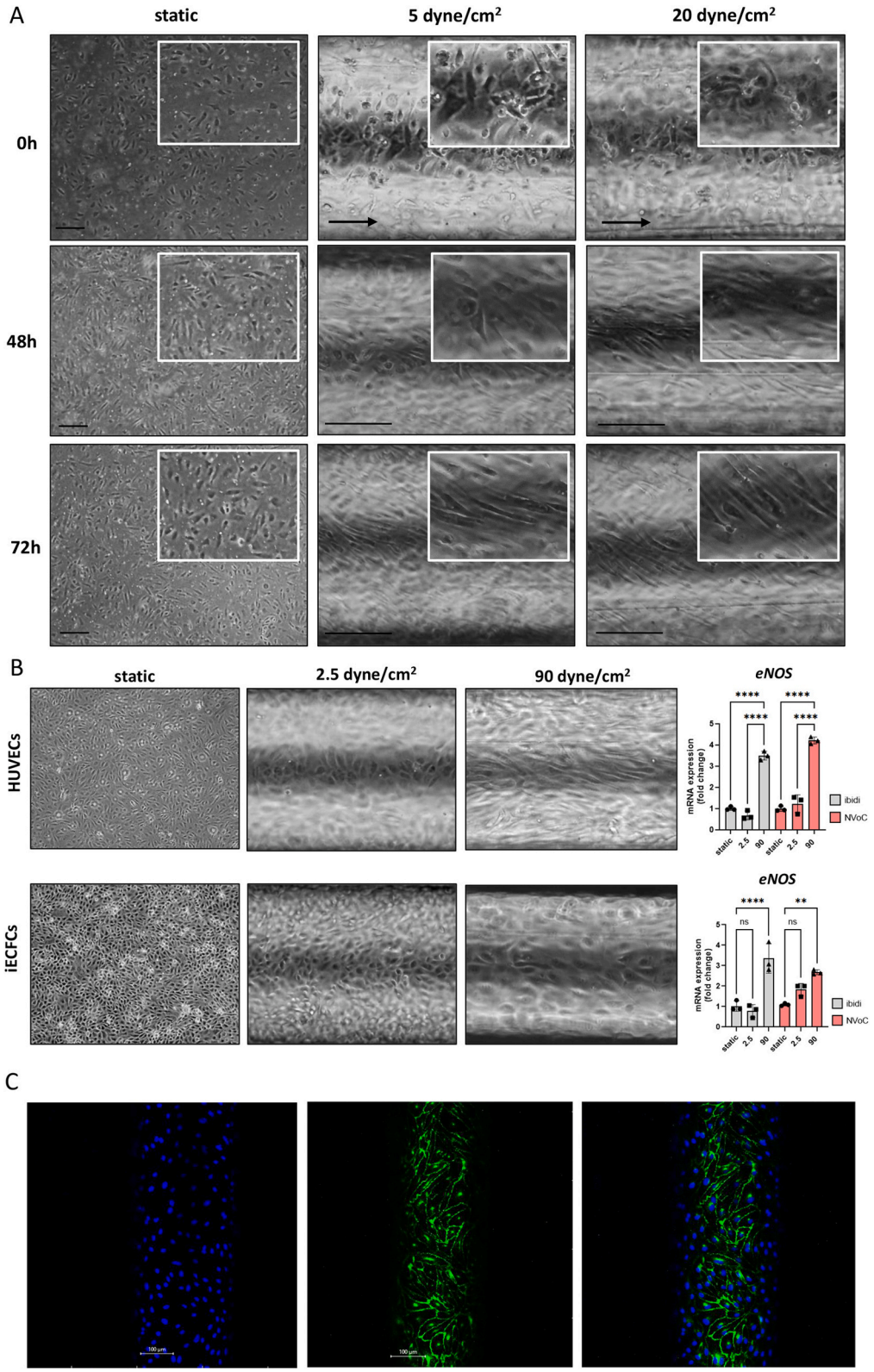

Fig. 8. Flow-induced shear alignment of human umbilical vein endothelial cells (HUVECs) under shear stress. (A) Representative brightfield images of HUVECs cultured under static conditions or exposed to shear stress levels of 5 and 20 dyne/cm^2 for 72 h. The scale bar represents 200 μm and the arrow indicates the direction of the flow. (A) Representative brightfield images of HUVECs cultured under static conditions or exposed to shear stress levels of 5 and 20 dyne/cm^2 for 72 h. The scale bar represents 200 μm and the arrow indicates the direction of the flow. (B) Brightfield images of HUVECs and iECFCs after 48 h under static conditions, 2.5 dyne/cm^2, or 90 dyne/cm^2 in the NVoC. The bar graph shows eNOS mRNA expression levels under the same conditions, comparing the NVoC (red bars) and Luer chip (grey bars). (C) Immunofluorescence staining of VE-Cadherin (green) and DAPI (blue) in HUVECs after exposure to shear stress, showing VE-Cadherin localization at cell–cell junctions. The scale bar represents 100 μm.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

The data supporting the findings of this study can be obtained from the corresponding authors upon reasonable request.

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