

YAP-antagonistic nanofiber-laminated tracheal stent abrogates in-stent restenosis pathological cascade

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Abstract

Tracheal in-stent restenosis (ISR) stems from a cascade of bacterial infection, inflammation, pathological angiogenesis and fibrosis, which cannot be synchronously targeted by conventional stents. Herein, electrospinning was used to fabricate a YAP-antagonistic nanofiber-laminated tracheal stent (PLVS) co-loaded with verteporfin and astragaloside IV, featuring uniform nanofibrous structure, robust mechanics and tunable dual-drug sustained release. In vitro tests confirm PLVS restrains MRSA proliferation, polarizes macrophages to anti-inflammatory M2 phenotype, suppresses pathological angiogenesis and fibroblast activation, and abrogates aberrant YAP signaling. In rabbit tracheal models, PLVS alleviates inflammation, reduces microvessel and collagen deposition, upregulates epithelial protective FOXJ1, and maintains tracheal patency. Collectively, the combined herbal monomer and YAP antagonist within PLVS fully disrupt the pathological cascade of ISR, representing a translatable candidate for tracheal stenosis treatment.

Introduction

Tracheal stenting is the primary palliative intervention for tracheal stenosis, yet post-procedural in-stent restenosis (ISR) caused by hyperplastic granulation tissue often occludes the tracheal lumen and compromises long-term clinical outcomes [1–3]. ISR arises from the synergistic cascade of bacterial colonization, inflammation, aberrant angiogenesis and fibrosis, while conventional single-drug stents fail to block this multifactorial pathological process [4, 5]. Bacterial biofilms shift macrophages to a pro-inflammatory M1 phenotype with persistent cytokine secretion, driving endothelial overgrowth, fibroblast activation and collagen deposition that ultimately induces luminal stenosis [6, 7]. Accordingly, developing multifunctional drug-eluting tracheal stents to simultaneously target multi-stage pathological cascades has become a core research focus in interventional pulmonology [8, 9].

As the primary downstream effector of the Hippo cascade, Yes-associated protein 1 (YAP) functions as a master signaling node interconnecting inflammatory response, pathological angiogenesis and fibrotic tissue remodeling [10–12]. Physiologically, intact Hippo signaling sequesters cytoplasmic YAP to sustain tracheal tissue homeostasis [13]. Upon pathological injury stimuli, YAP aberrantly translocates into the nucleus, where it complexes with TEAD transcription factors to upregulate pro-fibrotic and pro-angiogenic mediators, including CTGF and CYR61 [10, 14]. This transcriptional cascade accelerates fibroblast proliferation, myofibroblast differentiation and massive extracellular matrix deposition, thereby exacerbating scar hyperplasia and aggravating ISR [15, 16]. Substantial preclinical evidence confirms that sustained YAP hyperactivation contributes to fibrogenesis and pathological scarring across pulmonary, hepatic and other visceral organs, marking YAP as a compelling therapeutic target for anti-fibrotic intervention [17, 14, 18]. However, multifunctional tracheal stents engineered to concurrently suppress YAP-mediated fibrogenesis and relieve upstream bacterial infection and inflammation remain largely unreported.

To address this unmet research demand, two bioactive agents with complementary pharmacological functions were selected for combinatorial therapeutic design. Verteporfin (VP), a clinically licensed photosensitizer, specifically abrogates YAP–TEAD complex formation in a light-independent manner, which consequently represses fibroblast activation, extracellular matrix remodeling and pathological angiogenesis at the late fibrotic stage [19, 20]. In contrast, astragaloside IV (AS), a natural bioactive monomer purified from *Astragalus*, possesses robust antibacterial, anti-inflammatory and immunomodulatory capacities [21, 22]. AS suppresses the secretion of pro-inflammatory cytokines and remodels macrophage polarization from the pro-inflammatory M1 phenotype toward the reparative M2 phenotype, effectively alleviating the early inflammatory microenvironment induced by stent implantation [23]. Distinctly, AS intervenes at the initial infection-inflammation cascade, whereas VP exerts therapeutic effects on the terminal fibrotic stage, granting the two agents prominent functional complementarity [24, 25]. Their combinatorial application enables synergistic suppression of the stepwise pathological cascade after stent deployment, namely bacterial infection, persistent inflammation, aberrant YAP activation, pathological angiogenesis and progressive fibrosis [26, 27].

Herein, natural AS was extracted from *Astragalus* and blended together with VP in PLLA/HFIP spinning solutions. Electrospinning was adopted to fabricate nanofiber coatings for metallic stents, yielding the YAP-antagonistic nanofiber-laminated tracheal stent (PLVS) (**Schematic 1.**). Once implanted, the PLVS stent exerts four core protective effects on tracheal lesions by inhibiting MRSA colonization to provide antibacterial activity, polarizing macrophages toward the anti-inflammatory M2 phenotype to alleviate local inflammation, suppressing aberrant HUVEC proliferation to block pathological angiogenesis, and downregulating α -SMA and COL-1 expression to mitigate collagen-mediated fibrosis. Molecular mechanism studies revealed that AS and VP act through complementary signaling pathways: AS blocks upstream pathogenic NF- κ B, PI3K-AKT, and TGF- β cascades, while VP disrupts YAP–TEAD interaction to suppress downstream YAP signaling. Consistent with these mechanistic findings, in vivo experiments in New Zealand rabbits further validated that PLVS effectively abrogates the pathological cascade of ISR by curbing granulation overgrowth and sustaining unobstructed tracheal luminal patency.

Schematic 1. Schematic illustration showing the fabrication of YAP-antagonistic nanofiber-laminated tracheal stent and its multi-level mechanisms against in-stent tracheal stenosis.

Materials and Methods

2.1 Materials

Poly(L-lactic acid) (PLLA, Mw = 60,000) and hexafluoroisopropanol (HFIP) were purchased from Shanghai Macklin Biochemical Co., Ltd., while verteporfin (VP) was obtained from the same supplier. Astragaloside IV (AS) was sourced from Yuanye Biotechnology Co., Ltd. Self-expanding metal stents (SEMS, 20 mm $\times$ 8 mm) were supplied by Micro-Tech Medical Technology Co., Ltd. RAW 264.7 murine macrophages, human umbilical vein endothelial cells (HUVECs) and human pulmonary fibroblasts (HPFs) were acquired from the Cell Bank of the Shanghai Institutes for Biological Sciences, Chinese

Academy of Sciences. General cell culture reagents including DMEM, fetal bovine serum, penicillin–streptomycin and trypsin–EDTA were purchased from Solarbio and Sigma-Aldrich, while functional assay kits (CKK-8, live/dead cell staining, bacterial viability assay) were obtained from Dojindo, Thermo Fisher Scientific and Beyotime Biotechnology. Tracheal simulation fluid (TSF) was provided by Chuangfeng Automation Technology Co., Ltd., and methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33592) was adopted for antibacterial assessment. All chemical and biological reagents were of analytical grade and used without further purification.

2.2 Fabrication of YAP-Antagonistic Nanofiber-Laminated Tracheal Stents

A 12.5 wt% PLLA/HFIP base solution was prepared, followed by separate dissolution of VP and AS into the PLLA matrix to formulate PLLA/VP and PLLA/AS precursor solutions, which were further blended to generate the dual-drug PLLA/VP/AS (PLVS) spinning solution. Nanofibrous coatings were uniformly deposited onto fully expanded SEMS via electrospinning under fixed parameters: applied voltage of 15 kV, solution feeding rate of 0.5 mL/h, and 20 cm distance between the needle tip and rotary collector. Each stent was coated with 1.7 mL spinning solution to guarantee consistent membrane thickness, producing stents with uniform dimensions of 2 cm in length and 0.8 cm in diameter. Four experimental groups were defined based on drug loading formulations: pure PLLA, single VP-loaded PLVP, single AS-loaded PLAS, and dual-drug PLVS. All fabricated stents were fully dried, sealed and stored at 4°C for subsequent characterization and biological assays.

2.3 Physicochemical Characterization

The chemical composition of nanofibrous membranes was characterized via Fourier transform infrared spectroscopy (FTIR, Nicolet 5700, scanning range: 500–4000 cm^{-1}) and Raman spectroscopy (DXR2, Thermo Fisher Scientific). Surface micromorphology and elemental distribution were visualized by scanning electron microscopy (SEM, JSM-7401F, JEOL) coupled with energy-dispersive X-ray spectroscopy (EDS). Static water contact angle measurement based on the Laplace–Young method was performed to evaluate surface wettability using a contact angle goniometer. Tensile mechanical properties were tested on a universal testing machine (CMT6503, SANS) at a stretching rate of 10 mm/min to record tensile strength values. To assess coating flexibility suitable for tracheal implantation, rectangular nanofiber films were bent sequentially at 0°, 90°, 180° and 360°, and morphological integrity was photographed and recorded to verify structural stability under cyclic deformation.

2.4 In vitro Drug Release and Degradation Assays

For drug release evaluation, approximately 10 mg nanofibrous membrane fragments were immersed in 5 mL PBS or TSF and incubated at 37°C with continuous shaking at 50 rpm. At preset time points spanning 0–30 days, 1 mL release medium was collected for quantitative detection, with an equal volume of fresh medium replenished simultaneously to maintain a constant system volume. The absorbance of VP and AS was separately measured at 427 nm and 203 nm via UV–VIS spectrophotometry (Shimadzu 3700), and cumulative drug release ratios were calculated according to pre-established standard calibration

curves, with all time-point tests performed in biological triplicate. For in vitro degradation testing, 100 mg membrane specimens were submerged in PBS or TSF at 37°C under 80 rpm agitation, and the degradation medium was refreshed daily throughout the incubation period. At designated time intervals, samples were retrieved, rinsed three times with distilled water to remove residual salts and degradation debris, dried to constant weight and weighed. The degradation rate was calculated using the formula: Degradation Rate (%) = $[(W_0 - W_t) / W_0] \times 100\%$, where W_0 represents the initial dry mass and W_t denotes the residual dry mass at the corresponding time point.

2.5 In vitro Antibacterial Evaluation

All nanofibrous membranes were cut into 1 cm × 1 cm slices and sterilized under ultraviolet irradiation for 30 min prior to MRSA-related experiments, with six experimental groups set up including blank control (B.C.), positive control (P.C.), PLLA, PLVP, PLAS and PLVS. For bacterial proliferation assays, sterilized samples were placed in six-well plates and co-cultured with 2 mL bacterial liquid medium containing 20 µL MRSA suspension at 37°C for 24 h. 200 µL bacterial suspension was extracted at regular intervals to measure OD600 absorbance on a microplate reader for growth inhibition assessment. In colony formation assays, co-incubation lasted for 5 h before bacterial suspensions were evenly spread onto LB agar plates and cultured for another 16–24 h, after which colony-forming units (CFUs) were counted to calculate antibacterial inhibition rate (IR) via the formula $IR (\%) = [(N_0 - N) / N_0] \times 100\%$, where N_0 refers to the average CFU number of blank control and N represents the average CFU count of material co-culture groups.

For live/dead bacterial staining and SEM observation, membranes were incubated with MRSA suspension (1×10^8 CFU/mL) for 5 h, followed by DMAO/EthD-III fluorescent staining in dark at room temperature for 15 min. The stained bacterial samples were observed under a laser scanning confocal microscope (LCS-SP8-STED, Leica) to distinguish live and dead bacteria, while surface-adhered bacterial samples were fixed with 4% paraformaldehyde, subjected to gradient dehydration and imaged by SEM to capture morphological damage. An enhanced BCA protein leakage assay was conducted to detect bacterial membrane rupture: samples were co-cultured with MRSA suspension for 4 h at 37°C in a thermostatic shaking bath, culture supernatants were collected after centrifugation, and supernatant protein concentration was quantified by measuring OD562 absorbance to quantify intracellular component leakage induced by membrane damage.

2.6 In vitro Cellular Functional Assays

All nanofibrous membranes were cut into 1 cm × 1 cm pieces and UV-sterilized for 30 min before cell experiments, with five groups established: blank control (B.C.), PLLA, PLVP, PLAS and PLVS. RAW 264.7 macrophages were seeded on glass coverslips at a density of 1×10^5 cells/mL and treated with corresponding materials for 24 h to detect macrophage polarization via immunofluorescence staining targeting M1 marker iNOS and M2 markers CD206 and ARG-1. The cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, blocked with goat serum, incubated with

primary antibodies and Cy3-labeled secondary antibodies, and counterstained with DAPI before fluorescence imaging and quantitative intensity analysis using ImageJ software.

HUVECs and HPFs were separately utilized to assess anti-angiogenic and anti-fibrotic effects through a series of parallel assays. Cells were seeded into 96-well plates and cultured until attachment, followed by medium replacement with material-containing treatment solutions. CCK-8 reagent was supplemented on days 1, 2 and 3 of incubation, and OD450 absorbance was recorded after a further 3 h incubation to quantify cell viability. For live/dead cell staining, cells were seeded in 24-well plates and treated for 48 h, stained sequentially with Calcein-AM and propidium iodide (PI), and observed under an inverted fluorescence microscope (IX53, Olympus) to calculate live/dead cell ratios. Scratch wound-healing assays were carried out on fully confluent cell monolayers scratched with sterile pipette tips; detached cells were washed away with PBS, treatment medium was replenished, and scratch areas were photographed at 0 h and 24 h to compute wound closure rates via ImageJ. A tube formation assay was specifically applied to HUVECs: cells were seeded on pre-gelled Matrigel matrix and cultured for 24 h, after which capillary-like tubular networks were imaged and branch point numbers were quantified to evaluate angiogenic capacity. Universal immunofluorescence staining protocols were adopted for HUVEC and HPF biomarker detection, including fixation, permeabilization, blocking, primary antibody incubation against CD31, Ki67, COL-1, YAP and α -SMA, secondary antibody labeling and DAPI nuclear counterstaining, followed by fluorescence intensity quantification.

2.7 In vivo Tracheal Stent Implantation and Therapeutic Efficacy Assessment

All animal protocols were approved by the Animal Ethics Committee of the First Affiliated Hospital of Zhengzhou University and implemented in accordance with NIH laboratory animal care guidelines. Twenty male New Zealand Rabbits were randomly divided into four groups (PLLA, PLVP, PLAS, PLVS, n = 5 per group). Animals were intramuscularly anesthetized before surgery, placed in supine position with fully extended neck, and subjected to DSA fluoroscopic guidance (Artis zee, Siemens) for stent implantation via a 5 Fr delivery system. A 0.035-inch guidewire matched with a 5 Fr catheter was used to establish an intratracheal working channel, and stents were deployed 1.5–2.0 cm above the tracheal carina, followed by routine postoperative nursing. Four weeks after implantation, all rabbits received intramuscular xylazine hydrochloride anesthesia for cervical and thoracic CT scanning (Sensation 64, Siemens; slice thickness = 1 mm, interval = 0.5 mm). Multiplanar reconstruction (MPR) was performed on CT images to evaluate tracheal luminal patency, stent morphology and granulation tissue hyperplasia. After euthanasia, tracheal segments covering the stented region were harvested, fixed in 4% paraformaldehyde, paraffin-embedded and serially sectioned for histological staining. H&E and Masson's trichrome staining were conducted to quantify inflammatory infiltration and collagen deposition, while immunohistochemical staining was performed to detect the expression levels of IL-6, IL-10, Ki67, CD31, α -SMA and YAP in tracheal granulation tissues.

2.8 In vivo Degradation and Systemic Biosafety Evaluation

Rectangular nanofibrous membrane specimens (2 cm × 2 cm) were subcutaneously implanted in rats to investigate in vivo degradation behavior. Implantation sites with surrounding subcutaneous tissues were excised at days 7 and 28 post-operation, and tissue sections were subjected to H&E staining to observe membrane degradation and local inflammatory response. For systemic biosafety verification, heart, liver, spleen, lung and kidney tissues were isolated from all rabbit groups at the end of the 4-week experiment, processed for H&E staining to check visceral structural lesions, and serum samples were collected to detect routine hepatic and renal biochemical indicators.

2.9 Transcriptome Sequencing and Bioinformatic Analysis

Tracheal tissues from PLLA and PLVS groups were harvested, snap-frozen in liquid nitrogen and ground into fine powder. Total RNA was extracted using TRIzol reagent, and RNA concentration and purity were verified via UV spectrophotometry. Whole-transcriptome sequencing was commissioned to Wuhan GENOSEQ Biotechnology Co., Ltd. Differentially expressed genes screened from sequencing data were subjected to Gene Ontology (GO) enrichment analysis and Gene Set Enrichment Analysis (GSEA) to elucidate the core molecular regulatory mechanisms through which PLVS coating suppresses tracheal pathological remodeling.

2.10 Statistical Analysis

All quantitative data were expressed as mean ± standard deviation (Mean ± SD). Student's t-test was adopted for two-group comparisons, while one-way analysis of variance (one-way ANOVA) was used for multi-group statistical comparisons. All statistical calculations were completed on GraphPad Prism 8.0 software. Statistical significance thresholds were defined as N.S. (no significant difference), *P < 0.05, **P < 0.01 and ***P < 0.001.

Results

3.1 Physicochemical properties, degradation and drug-release profiles

Fourier transform infrared (FTIR) spectroscopy was first utilized to verify the chemical composition of composite nanofibers (Fig. 1a,b). Pure PLLA, VP, and AS displayed distinct characteristic absorption peaks [28, 29]. All drug-loaded groups (PLVP, PLAS, and PLVS) retained the intrinsic characteristic peaks of PLLA matrix, with only minor shifts in drug-specific signals, demonstrating successful drug encapsulation without disruption of the PLLA chemical backbone. Raman spectroscopy further validated the FTIR findings (**Figure S1**, Fig. 1c,d). The co-doped PLVS fibers presented complete Raman signatures of PLLA, VP, and AS, confirming the homogeneous and stable incorporation of dual drugs within the fibrous network [30, 31]. Scanning electron microscopy (SEM) observations showed that all groups yielded continuous, uniform, and three-dimensionally interconnected nanofibrous networks without beads, fractures, or other structural defects (Fig. 1e). Statistical diameter analysis revealed an average fiber size of 424–567 nm across all groups (Fig. 1g), indicating that VP and AS incorporation exerted no

adverse effects on the electrospinning process. Energy-dispersive X-ray spectroscopy (EDS) mapping illustrated homogeneous distribution of carbon, oxygen, and nitrogen elements in PLVS fibers (Fig. 1f,h). The nitrogen originating from VP exhibited no localized aggregation, further verifying the uniform dispersion of dual active ingredients in the nanofibers, consistent with spectroscopic results.

Mechanical tests demonstrated that single- and dual-drug modification effectively improved the mechanical properties of PLLA nanofibers, with PLVS dual-drug membranes exhibiting the optimal tensile strength (Fig. 1i), which reflected enhanced structural stability via the co-loading strategy. Water contact angle tests confirmed the highly hydrophobic surface of all samples. The introduction of VP and AS only slightly reduced the contact angle, without significantly altering the surface wettability of the PLLA substrate (Fig. 1j). Collectively, these results confirm the successful fabrication of VP/AS dual-drug-loaded PLVS nanofibrous membranes. The composite material retained the inherent chemical structure and well-defined microarchitecture of PLLA, accompanied by favorable mechanical strength and stable hydrophobicity. These superior physicochemical properties provide a reliable material foundation for sustained local drug delivery and optimal in vivo biological performance of the stent system.

Controllable degradation and sustained drug release are essential guarantees for the long-term therapeutic efficacy of tracheal stents [32, 33, 2]. Herein, the in vitro degradation behavior and drug release kinetics of engineered nanofibrous membranes were systematically investigated in PBS and tissue simulating fluid (TSF). The degradation results revealed a time-dependent mass loss for all membrane groups. Compared with pure PLLA, single and dual drug-loaded membranes exhibited slightly accelerated degradation rates, while no obvious discrepancy in degradation trends was observed between PBS and TSF media (Fig. 1k). Such progressive and controllable degradation behavior enables the scaffolds to provide stable early structural support and subsequent gradual material erosion, satisfying the fundamental requirements for long-term local drug delivery.

In vitro drug release tests demonstrated that both VP and AS presented a typical biphasic release pattern from PLVS membranes, including an initial burst release within 0–10 days and a prolonged sustained release spanning 11–30 days (Fig. 1l). In PBS, the cumulative release of VP and AS from PLVS membranes reached $41.29\% \pm 4.42\%$ and $36.61\% \pm 1.58\%$ in the first 10 days, respectively, with the 30-day total cumulative release approaching 54%. No significant differences in overall release levels were observed between single-drug and dual-drug loaded groups. Moreover, release profiles acquired in TSF were highly consistent with those in PBS, verifying the excellent environmental adaptability and structural stability of the PLLA nanofibrous carrier. Collectively, the PLVS system achieves synchronous, stable, and prolonged co-release of VP and AS, which enables persistent regulation of the local inflammatory microenvironment and effective suppression of aberrant fibroblast proliferation. This durable and steady drug delivery capability can effectively block the pathological cascade after stent implantation, thereby mitigating airway stenosis and laying a solid foundation for long-term stent therapeutic performance.

3.2 In vitro antibacterial and anti-inflammatory performance

Bacterial infection and sustained inflammation after tracheal stent implantation are primary drivers of excessive granulation hyperplasia and subsequent airway restenosis [34–36]. Accordingly, we systematically evaluated the in vitro antibacterial and immunomodulatory capacities of the composite nanofibrous membranes, focusing on anti-MRSA activity and macrophage polarization regulation (Fig. 2a).

The proliferation kinetics of MRSA were dynamically monitored via OD600 absorbance (Fig. 2b). The blank, pure PLLA, and PLVP groups exhibited consistent logarithmic bacterial growth, indicating negligible antibacterial activity of pristine PLLA and single VP loading under dark conditions. In contrast, PLAS and PLVS groups significantly suppressed MRSA proliferation within 6 h of incubation. SEM visualization further verified distinct bacterial morphological alterations across groups (Fig. 2c). MRSA in the blank, PLLA, and PLVP groups retained intact spherical morphology, while bacteria cultured on PLAS and PLVS membranes presented severe shrinkage, structural collapse, and membrane damage. Quantitative colony formation assays validated the above antibacterial differences (Fig. 2d,f). The bacterial inhibition rates of PLAS and PLVS reached $83.0\% \pm 2.8\%$ and $90.1\% \pm 1.7\%$, respectively. Consistent with these results, live/dead staining revealed prominent red fluorescence (dead bacteria) and attenuated green fluorescence (live bacteria) in PLAS and PLVS groups (Fig. 2e,g). Moreover, the elevated extracellular protein leakage detected via BCA quantification confirmed severe bacterial membrane disruption induced by AS release (Fig. 2h), which triggered the leakage of intracellular components and eventual bacterial death.

Persistent bacterial infection polarizes macrophages toward the pro-inflammatory M1 phenotype, further exacerbating local inflammatory cascades and tissue hyperplasia [37, 38, 27]. As shown in Fig. 2i, LPS stimulation induced RAW264.7 cells to exhibit an M1 phenotype with branched pseudopodia, whereas PLVS treatment converted cells to a spindle shape with fewer pseudopodia, presenting typical characteristics of M2 phenotype. Immunofluorescence staining and quantitative fluorescence analysis of macrophage polarization markers were performed to further verify the regulatory effect (Fig. 2j). LPS stimulation significantly upregulated the expression of the M1 marker iNOS (Fig. 2k). Both PLVP and PLAS effectively inhibited iNOS expression, with PLVS exhibiting the most prominent suppressive effect. By contrast, LPS remarkably downregulated the M2 markers CD206 and ARG-1, while PLVP, PLAS, and particularly PLVS treatment significantly restored their expression levels (Fig. 2l,m). The PLVS efficiently inhibit bacterial proliferation and drive macrophage phenotypic transition from pro-inflammatory M1 to anti-inflammatory M2, thereby blocking the upstream pathological cascade and remodeling a favorable anti-inflammatory microenvironment for tracheal stent implantation.

3.3 In vitro anti-angiogenic and endothelial-suppressive effects

Pathological angiogenesis is a central contributor to post-stent airway restenosis, which sustains granulation tissue hyperplasia, exacerbates maladaptive airway remodeling, and perpetuates local inflammatory microenvironments by delivering abundant nutrients to injured lesions [39–41]. Targeting aberrant endothelial angiogenesis therefore represents a core therapeutic strategy against fibrotic airway stenosis. Herein, human umbilical vein endothelial cells (HUVECs) were employed to systematically evaluate the anti-angiogenic and endothelial-inhibitory capacities of engineered nanofibrous membranes (Fig. 3a).

CCK-8 assays demonstrated robust and comparable proliferation of HUVECs in blank and pure PLLA groups, validating the favorable endothelial biocompatibility and biological inertness of the PLLA matrix (Fig. 3b). In contrast, PLVP and PLVS membranes exerted persistent and pronounced suppression on HUVECs proliferation throughout 1–3 days of culture, whereas PLAS treatment showed negligible inhibitory effects. Calcein-AM/PI live/dead staining further corroborated these phenotypic differences (Fig. 3c,d). B.C., PLLA, and PLAS groups retained predominant viable endothelial cells, while PLVP and PLVS groups exhibited markedly increased dead cell signals, confirming that sustained VP release effectively impairs endothelial survival. Scratch wound healing assays were performed to assess the effect of each material group on the migratory capacity of HUVECs (Fig. 3e, g). After 24h of incubation, the scratched areas in B.C. and PLLA groups were almost completely closed. In contrast, cell migration was markedly restricted in PLVP and PLVS groups, with only a small number of cells observed migrating into the scratched regions. PLAS group exhibited a weak inhibitory effect on cell migration. Matrigel tube formation assays were conducted to mimic in vitro angiogenesis (Fig. 3e, h). HUVECs from B.C. and PLLA groups formed full, connected capillary-like tubes. By contrast, cells treated with PLVP or PLVS could not form intact tubes. They appeared as scattered cells or tiny clusters, with far fewer branch points. This indicates VP-containing nanofibers strongly inhibit endothelial angiogenesis. Immunofluorescence staining of endothelial biomarkers provided molecular evidence for endothelial repression (Fig. 3f,i,j). B.C. and PLLA groups presented high expression levels of the endothelial lineage marker CD31 and proliferation marker Ki67. Conversely, PLVP and PLVS treatment significantly downregulated the fluorescence intensities of both markers, demonstrating that VP-loaded nanofibers efficiently inhibit endothelial proliferation and aberrant endothelialization.

3.4 PLVS exert anti-fibrotic effects by ablating YAP signaling

Pathological fibrosis driven by unrestrained fibroblast proliferation, migration and aberrant myofibroblast conversion represents the core pathogenic driver of post-stent airway stenosis [18, 42]. As the chief transcriptional mediator of the Hippo cascade, YAP governs fibroblast activation, excessive collagen deposition and progressive airway structural remodeling [43, 44]. Human primary pulmonary fibroblasts (HPFs) were adopted as an in vitro fibrotic model to probe how our drug-coated stent-targeted nanofibrous scaffolds reshape pathological fibroblast phenotypes alongside YAP signal transduction (Fig. 4a).

Mirroring earlier endothelial cell assessments, CCK-8 and Calcein-AM/PI live-dead profiling validated PLLA's favorable cytocompatibility. VP-containing PLVP and PLVS sharply curbed HPF viability across three days of incubation, whereas PLAS only weakly restricted fibroblast growth (Fig. 4b–d). Scratch wound assays further revealed that dual-drug PLVS outperformed single-drug groups in hindering fibroblast motility (Fig. 4e,h). Immunofluorescent detection of Ki67 and COL-1 recapitulated this synergistic tendency: all drug-loaded scaffolds mitigated proliferative and matrix-synthetic signatures, with PLVS producing the strongest inhibitory phenotype (Fig. 4f,i,j).

Myofibroblast transdifferentiation constitutes an irreversible checkpoint during fibrotic progression [45]. To decipher the molecular mechanism behind fibrosis suppression mediated by this YAP-targeted stent coating, we performed immunolabeling for the myofibroblast biomarker α -SMA and its upstream profibrotic regulator YAP (Fig. 4g,k,l). All drug-functionalized membranes downregulated α -SMA abundance, and PLVS achieved complete abrogation of fibroblast-to-myofibroblast phenotypic switching. Abundant YAP protein accumulated in blank, PLLA and PLAS groups; in contrast, PLVP and PLVS drastically erased YAP fluorescent signals, and the dual-drug scaffold exhibited the most potent capacity for ablating YAP signaling.

Cumulatively, these cellular data verify that VP selectively antagonizes YAP cascade activation to suppress downstream profibrotic mediators α -SMA and COL-1. When co-delivered with AS, VP generates synergistic anti-fibrotic biofunction by simultaneously restraining fibroblast proliferation, migration and myofibroblast reprogramming. This YAP-targeted dual-drug nanofibrous coating therefore endows metallic tracheal stents with prominent in vitro anti-fibrotic capacity to alleviate stent-induced airway fibrotic stenosis.

3.5 In vivo adaptability and therapeutic efficacy against airway stenosis

Functional tracheal stent coatings require superior mechanical compliance and tunable biodegradation to accommodate physiological tracheal deformation, alleviate persistent foreign-body irritation, and sustain localized cargo delivery [46, 47]. Cyclic bending assessments validated the exceptional structural resilience of composite nanofibrous films. All groups maintained intact architecture after successive deformation at 0°, 90°, 180° and 360°, devoid of cracking, rupture or interfacial delamination (Fig. 5a). Such robust mechanical endurance satisfies the dynamic biomechanical demands of tracheal implantation. In vivo degradation and histocompatibility evaluation revealed gradual, sustained erosion of the nanofibrous coating post-implantation (Fig. 5b). At day 28, peritracheal tissue exhibited negligible foreign-body giant cell infiltration and muted inflammatory infiltration, substantiating the outstanding biosafety of the YAP-antagonistic nanofiber-laminated tracheal stent.

A rabbit tracheal stenting model was established to interrogate the in vivo anti-restenosis potency (Fig. 5c). Digital subtraction angiography (DSA) enabled precise navigation and accurate deployment of all stents within target tracheal segments (Fig. 5d). Four weeks post-intervention, sagittal CT

tomography and three-dimensional tracheal reconstructions revealed distinct luminal remodeling disparities across cohorts (Fig. 5f). The PLLA control group suffered severe luminal occlusion triggered by rampant granulation hyperplasia. Monotherapy PLVP and PLAS coatings partially mitigated intraluminal tissue overgrowth yet failed to avert substantial stenosis, whereas the dual-agent PLVS coating drastically curtailed granulation ingrowth and preserved optimal tracheal patency. Quantitative ventilation rate analysis corroborated this phenotypic hierarchy (Fig. 5h). All drug-loaded formulations conferred superior ventilatory patency relative to blank PLLA, with PLVS delivering maximal luminal protection. Gross anatomical inspection of harvested tracheal specimens further validated this trend, as PLVS displayed the minimal intrastent neotissue coverage (Fig. 5g).

Histopathological profiling quantified tracheal mucosal injury and fibrotic burden microscopically (Fig. 5e). H&E staining identified drastic tracheal wall thickening and exuberant granulation deposition in PLLA samples. These pathological lesions were moderately alleviated by PLVP and PLAS, while PLVS largely conserved native tracheal histoarchitecture with markedly attenuated granulation proliferation. Masson's trichrome staining visualized pervasive pathological collagen aggregation within PLLA tracheas, a phenotype profoundly abrogated in the PLVS cohort. Morphometric quantification demonstrated epithelial hyperplasia thickness of $1235.47 \pm 103.85 \mu\text{m}$ in PLLA, sharply diminished to $481.07 \pm 122.67 \mu\text{m}$ upon PLVS treatment (Fig. 5i). The collagen volume fraction fell from $31.54\% \pm 4.62\%$ (PLLA) to $18.03\% \pm 4.62\%$ (PLVP), $16.53\% \pm 2.53\%$ (PLAS), and merely $9.00\% \pm 0.67\%$ for PLVS (Fig. 5j). These in vivo phenotypic evidence solidifies that this stent potently represses granulation overgrowth and aberrant collagen matrix deposition, sustaining durable tracheal luminal patency and conferring robust therapeutic efficacy to counteract post-stenting tracheal restenosis.

3.6 In vivo synergistic triple therapeutic activities

To dissect the core in vivo mechanism underlying the anti-stenotic efficacy, tracheal tissues harvested at week 4 post-implantation were subjected to multiplex histological profiling targeting inflammatory, angiogenic and profibrotic signaling signatures. Immunohistochemical quantification of pro-inflammatory IL-6 uncovered abundant positive staining occupying $24.51\% \pm 1.13\%$ of tissue area within the PLLA cohort. While monotherapeutic PLVP and PLAS each attenuated IL-6 abundance moderately, PLVS elicited maximal repression, restricting IL-6-positive regions to merely $3.55\% \pm 1.38\%$ (Fig. 6a,b). Complementary IL-10 immunolabeling revealed the most robust anti-inflammatory cytokine enrichment in PLVS-treated tracheas, indicative of restored homeostatic persistent inflammatory microenvironment (Fig. 6c).

Dual immunofluorescent labeling of endothelial marker CD31 and proliferation biomarker Ki67 was implemented to quantify intraluminal neovascularization and proliferative activity (Fig. 6d–f). PLLA samples exhibited dense CD31⁺ microvessels and Ki67⁺ proliferative populations, signifying robust pathological angiogenic remodeling. Partial neovascular restraint was observed in PLVP and PLAS groups; conversely, PLVS yielded the faintest CD31 and Ki67 fluorescent signals, verifying potent in vivo blockade of aberrant angiogenesis and cellular overgrowth.

Fibrotic pathway assessment demonstrated extensive α -SMA immunoreactivity ($31.64\% \pm 0.86\%$) in PLLA tracheas, a hallmark of exacerbated myofibroblast transdifferentiation and fibrotic lesion progression (Fig. 6g–i). Single-drug formulations conferred mild fibrotic mitigation, yet PLVS sharply curtailed α -SMA-positive tissue area to $9.13\% \pm 0.97\%$. Elevated YAP abundance was detected in PLLA and PLAS cohorts, whereas PLVS markedly blunted YAP tissue occupancy to $7.58\% \pm 1.16\%$, corroborating that this dual-agent stent coating halts fibrotic advancement and myofibroblast activation via selective YAP cascade abrogation. Immunofluorescence staining and quantitative analysis of the ciliated epithelial differentiation marker FOXJ1 were performed (Fig. 6j, k). The PLLA group exhibited only faint FOXJ1 fluorescence signals, while the signals were moderately elevated in the PLVP and PLAS groups. The PLVS group showed the strongest FOXJ1 fluorescence intensity among all groups, indicating robust ciliated epithelial differentiation. These results confirm that the dual-drug stent not only exerts anti-fibrotic effects but also facilitates the regeneration of epithelial cilia and the repair of mucosal function.

Comprehensive systemic biosafety validation confirmed all routine hematological, hepatic and renal biochemical indices stayed within physiological reference ranges across all experimental groups at postoperative week 4 (**Figure S2a–h**). H&E histology of major visceral organs (heart, liver, spleen, lung, kidney) displayed no detectable structural injury or inflammatory lesions, consolidating the outstanding systemic histocompatibility and in vivo biosafety (**Figure S2i**).

3.7 Transcriptomic mechanism/basis of PLVS-suppressed airway remodeling

RNA-seq profiling was performed on tracheal tissues harvested 4 weeks post-implantation from PLLA and PLVS groups to decode the transcriptomic landscape underlying stent-mediated anti-stenotic effects. The volcano plot identified 3322 upregulated and 1830 downregulated transcripts in PLVS relative to PLLA (Fig. 7a), while hierarchical clustering heatmaps displayed distinct transcriptional signatures between the two cohorts (Fig. 7b). Profibrotic, pro-inflammatory and matrix-remodeling genes (MMP1/10/13, CXCR1/2, YAP1) were robustly repressed in PLVS specimens, whereas transcripts governing epithelial integrity and pulmonary homeostasis (IL36A, CIDEA, SFTPA1, CYP2F1, CYP2A10) were drastically elevated, indicative of a reparative transcriptional shift driven by the dual-drug coating.

GO enrichment of downregulated genes highlighted biological processes linked to cell adhesion, cytoskeletal rearrangement and extracellular matrix assembly, consistent with restrained fibroblast motility and tissue overgrowth (Fig. 7c,d). KEGG pathway enrichment pinpointed suppression of PI3K-Akt, focal adhesion, TGF- β , NF- κ B and IL-17 cascades, core signaling axes governing inflammation, angiogenesis and fibrotic progression (Fig. 7e,f). GSEA further corroborated this regulatory pattern: gene signatures associated with inflammation, pathological angiogenesis and epithelial-mesenchymal transition (EMT) were significantly depleted in the PLVS group (Fig. 7g).

Relative transcriptional abundance of representative hub genes was extracted from RNA-seq datasets for comparative visualization (Fig. 7h). PLVS treatment markedly lowered transcript levels of profibrotic YAP1, matrix metalloproteinases MMP1/MMP13 and pro-inflammatory chemokine CXCL5, while substantially elevating FOXJ1, a master transcription factor maintaining tracheal epithelial barrier stability [48, 49]. Collectively, transcriptomic evidence verifies that this YAP-axis-targeted therapeutic stent coating orchestrates multi-pathway synergistic modulation of inflammatory, angiogenic and profibrotic signaling networks, arresting excessive granulation hyperplasia at the transcriptional level to counteract post-stent airway restenosis.

Discussion

To break this pathological cascade and overcome limitations of existing therapies, we developed a YAP-antagonistic nanofiber-laminated tracheal stent co-loaded with natural astragaloside IV (AS) and YAP inhibitor verteporfin (VP). Conventional drug-eluting stents rely on cytotoxic paclitaxel or rapamycin [50–52], which only suppress cell proliferation without antibacterial or immunomodulatory effects, failing to block infection-initiated pathological progression. AS inhibits MRSA proliferation and polarizes macrophages toward anti-inflammatory M2 phenotypes to restrain early lesions, while VP selectively antagonizes YAP-TEAD to halt fibroblast activation and matrix accumulation in late fibrosis [53, 54]. This temporally matched dual-agent combination greatly improves anti-restenosis efficacy, yet advanced coating fabrication is required to maximize their in vivo multi-stage synergism.

Clinically conventional dip-coating and ultrasonic spraying for drug-eluting stents incur limited drug loading, poor coating stability and erratic release kinetics [55, 2]. Paclitaxel stents prepared by such methods show either insufficient long-term elution (22.85% over 90 days) [56] or drastic early burst release (> 70.9% within one month) [57], mismatching the temporal progression of tissue hyperplasia. Herein, electrospinning yielded uniform nanofibrous PLVS coatings; their large specific surface area and degradable polymer matrix enabled favorable biphasic sustained release in tracheal simulated fluid (TSF): rapid elution in the first 10 days to resolve early infection and inflammation, followed by prolonged slow release from day 11–30 for persistent anti-fibrotic activity. This profile sustains therapeutic local drug concentrations and underpins robust in vivo anti-restenosis capacity. Bare SEMS and uncoated covered stents commonly trigger severe granulation overgrowth post-implantation [58], with Li et al. recording epithelial hyperplasia up to $774.00 \pm 24.52 \mu\text{m}$ in bare stent groups [59]. By interrupting the pathological cascade, PLVS coatings remarkably curbed epithelial thickening and collagen deposition to $481.07 \pm 122.67 \mu\text{m}$ and $9.00\% \pm 0.67\%$, respectively.

Despite the robust therapeutic performance of PLVS identified in this work, several limitations persist. Evaluations were restricted to a 4-week implantation window, prohibiting thorough assessment of long-term coating degradation and durable efficacy. While RNA-seq pinpointed multiple regulatory cascades, interactions among core hub molecules demand further validation through Western blot, RT-qPCR and YAP nuclear translocation tests. Additionally, the rabbit model cannot fully recapitulate intricate human

airway lesions, necessitating follow-up large-animal trials to verify its long-term biosafety and clinical translatability.

Conclusion

- Herein, we fabricated electrospun PLLA nanofibrous PLVS coatings to remodel the peri-stent pathological microenvironment. Distinct from single-target antiproliferative stents, PLVS concurrently mitigates all core drivers of ISR: infection, inflammation, pathological angiogenesis and fibrosis. AS combats early bacterial invasion and inflammatory activation, while VP blocks YAP signaling to ablate late fibrotic remodeling. Spatiotemporally coordinated dual-agent synergism fully disrupts the ISR pathological cascade. Integrated in vitro, in vivo and transcriptomic characterizations confirm PLVS potently inhibits granulation overgrowth and preserves tracheal patency. This work broadens the translational scope of YAP antagonists for implantable devices and delivers an innovative blueprint for multifunctional drug-eluting stents targeting airway stenosis.

Declarations

Declarations

The authors have no financial or proprietary interests in any material discussed in this article.

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Author Contribution

Y.L. and J.Z. contributed equally to this work. Y.L. and J.Z. conceived the study, designed and performed the experiments, analyzed the data, and wrote the original manuscript. J.S., S.H., and Z.L. contributed to the experiments, data collection, and data analysis. Z.W. contributed to study design, data interpretation, and manuscript revision. X.H., K.R., Z.W., and Y.Z. supervised the study, provided critical intellectual input, and revised the manuscript. Y.Z. contributed to project administration and overall coordination. All authors reviewed and approved the final manuscript.

Data Availability

The datasets generated during the current study are not publicly available but are available from the corresponding author upon reasonable request.

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Figures

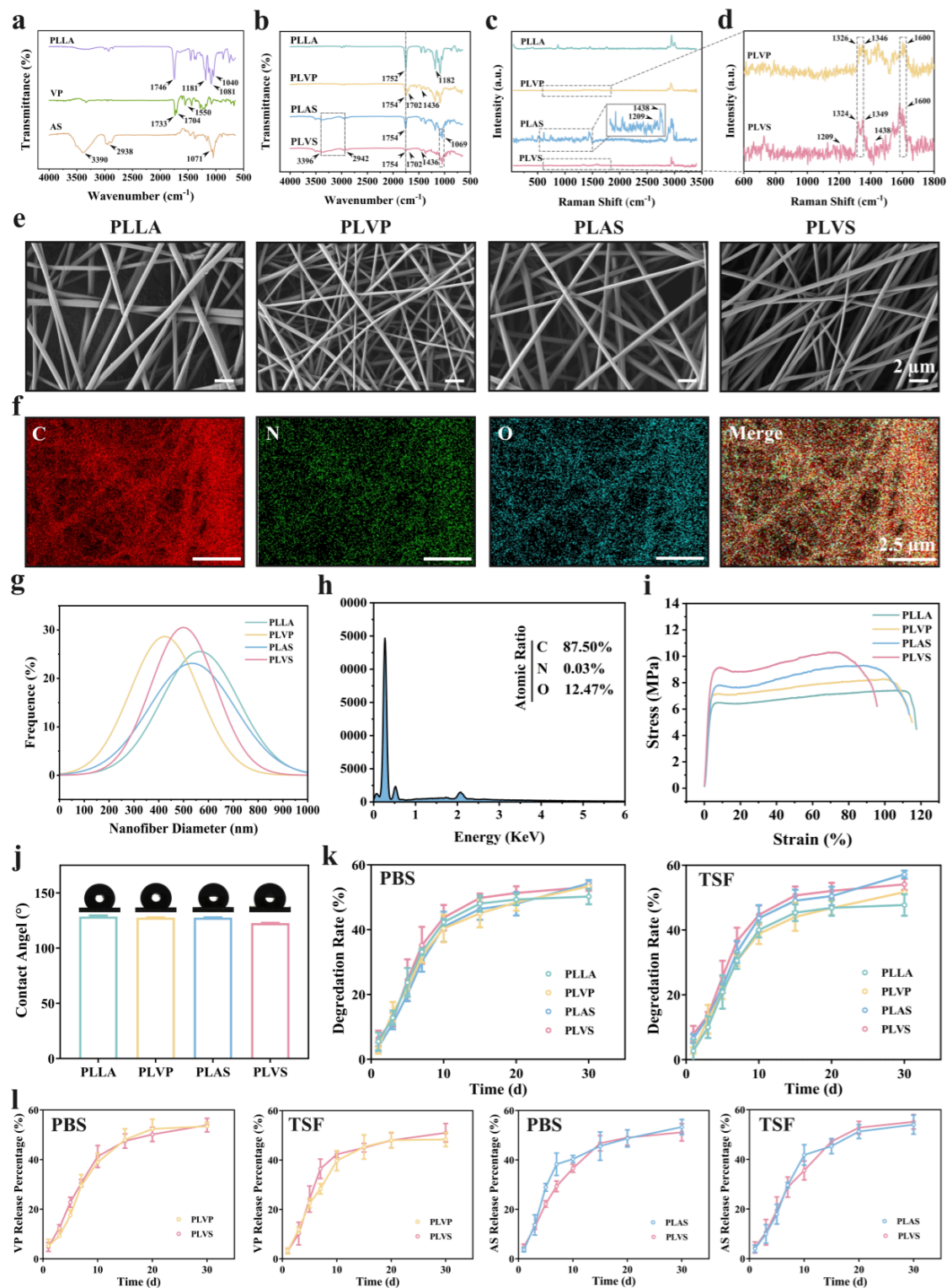


Figure 1

Fabrication, physicochemical characterization, in vitro degradation and synchronized dual-drug release profiles. (a) FTIR absorption spectra of pristine PLLA, free VP and free AS powders. (b) FTIR spectra of pure PLLA, single-drug loaded PLVP/PLAS and dual-drug loaded PLVS nanofibrous membranes. (c) Full-range Raman spectra of PLLA, PLVP, PLAS and PLVS nanofibers; inset shows magnified characteristic Raman peaks. (d) Magnified Raman spectral region (600–1800 cm^{-1}) comparing PLVP and PLVS groups

to identify characteristic signals of VP and AS. (e) SEM micrographs showing uniform interconnected nanofibrous networks of PLLA, PLVP, PLAS and PLVS; scale bar = 2 μm . (f) EDS elemental mapping of carbon (C), nitrogen (N), oxygen (O) and merged overlay for PLVS membrane, confirming homogeneous element distribution; scale bar = 2.5 μm . (g) Statistical frequency distribution of nanofiber diameters across all four experimental groups. (h) EDS energy spectrum and corresponding atomic ratio of C, N, O elements of PLVS nanofibers. (i) Tensile stress–strain curves of PLLA, PLVP, PLAS and PLVS nanofibrous membranes. (j) Quantitative water contact angle measurements for all membrane groups, with representative optical images of water droplets shown above each bar. (k) Time-dependent in vitro mass loss degradation curves of four groups incubated in PBS and TSF media over 30 days. (l) Cumulative release percentage of VP (from PLVP and PLVS) and AS (from PLAS and PLVS) within 30 days in PBS and TSF, revealing biphasic synchronous release behavior of the two cargos in the dual-drug system. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

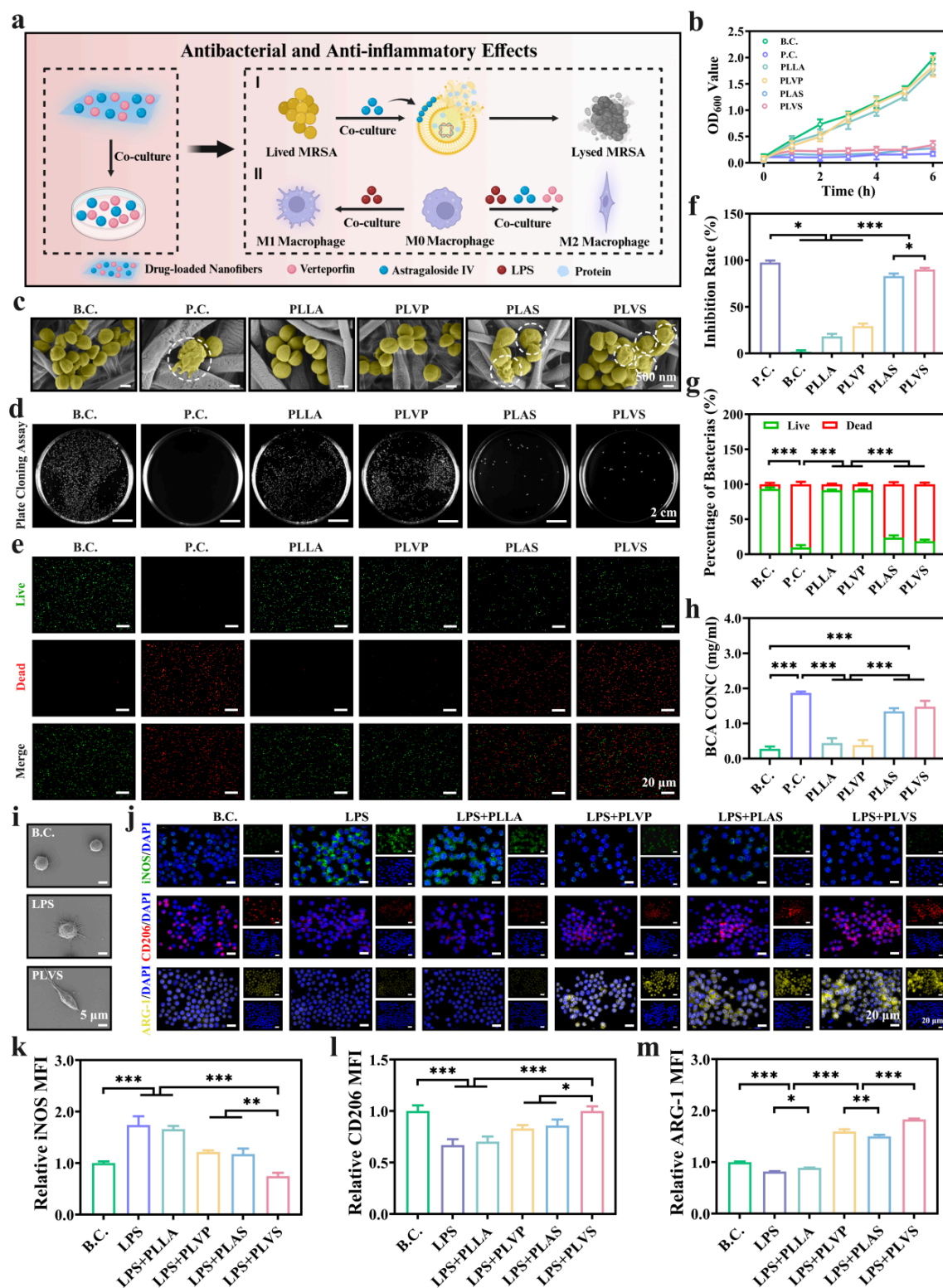


Figure 2

In vitro antibacterial and immunomodulatory functions. (a) Schematic illustration of the experimental design for evaluating anti-MRSA activity and macrophage polarization regulation. Process I depicts AS-mediated lysis of MRSA; Process II shows the phenotypic transformation of LPS-stimulated M1 macrophages toward anti-inflammatory M2 macrophages under dual-drug nanofiber treatment. (b) Time-dependent OD600 growth curves reflecting MRSA proliferation across all experimental groups. (c) SEM

micrographs of MRSA adhered to the surface of different nanofibrous membranes, scale bar: 500 nm. (d) Photographs of MRSA colony-forming unit (CFU) plate assays, scale bar: 2 cm. (e) Live/dead bacterial fluorescence staining images, live bacteria stained green and dead bacteria stained red, scale bar: 20 μ m. (f) Statistical quantification of antibacterial inhibition rates against MRSA for each group. (g) Quantitative proportion statistics of live and dead MRSA derived from live/dead staining. (h) BCA quantitative detection of leaked intracellular protein in MRSA culture supernatants. (i) SEM images displaying distinct morphological phenotypes of RAW264.7 macrophages under blank control (B.C.), LPS stimulation, and LPS+PLVS treatment, scale bar: 5 μ m. (j) Immunofluorescence staining of macrophage polarization biomarkers: M1 marker iNOS, M2 markers CD206 and Arg-1; nuclei counterstained with DAPI, scale bar: 20 μ m. (k–m) Quantitative analysis of relative mean fluorescence intensity (MFI) for iNOS, CD206 and Arg-1, respectively. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

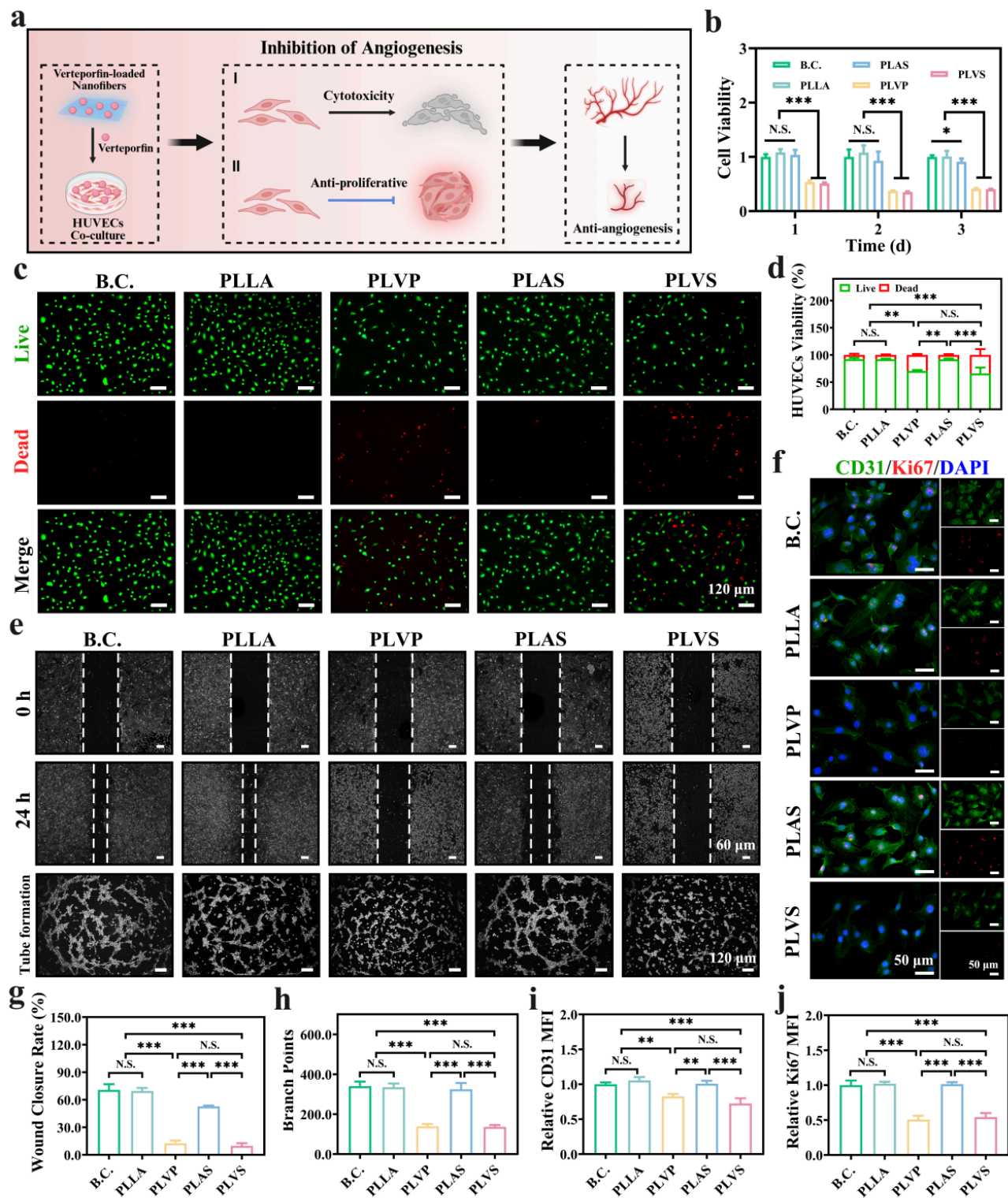


Figure 3

In vitro anti-angiogenic and endothelial-suppressive effects. (a) Schematic diagram illustrating the experimental paradigm for evaluating VP-mediated cytotoxicity, anti-proliferation and anti-angiogenic responses in HUVECs co-cultured with drug-loaded nanofibers. (b) Quantitative CCK-8 cell viability curves of HUVECs cultured with different nanofibrous membranes over 1, 2 and 3 days. (c) Representative Calcein-AM/PI live/dead fluorescence staining images of HUVECs under distinct

treatments, scale bar = 120 μm . (d) Statistical quantification of the percentage of live and dead endothelial cells based on live/dead staining. (e) Bright-field images of scratch wound healing at 0 h and 24 h and Matrigel tube formation networks of HUVECs across all groups, scale bars: 60 μm (scratch assay), 120 μm (tube formation assay). (f) Immunofluorescence staining of endothelial marker CD31 (green), proliferation marker Ki67 (red) and nuclear counterstain DAPI (blue) in HUVECs, scale bar = 50 μm . (g) Quantitative statistics of relative wound closure rates after 24 h incubation. (h) Quantification of capillary branch point numbers from tube formation images. (i,j) Relative mean fluorescence intensity (MFI) analysis of CD31 and Ki67, respectively. N.S., not significant. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

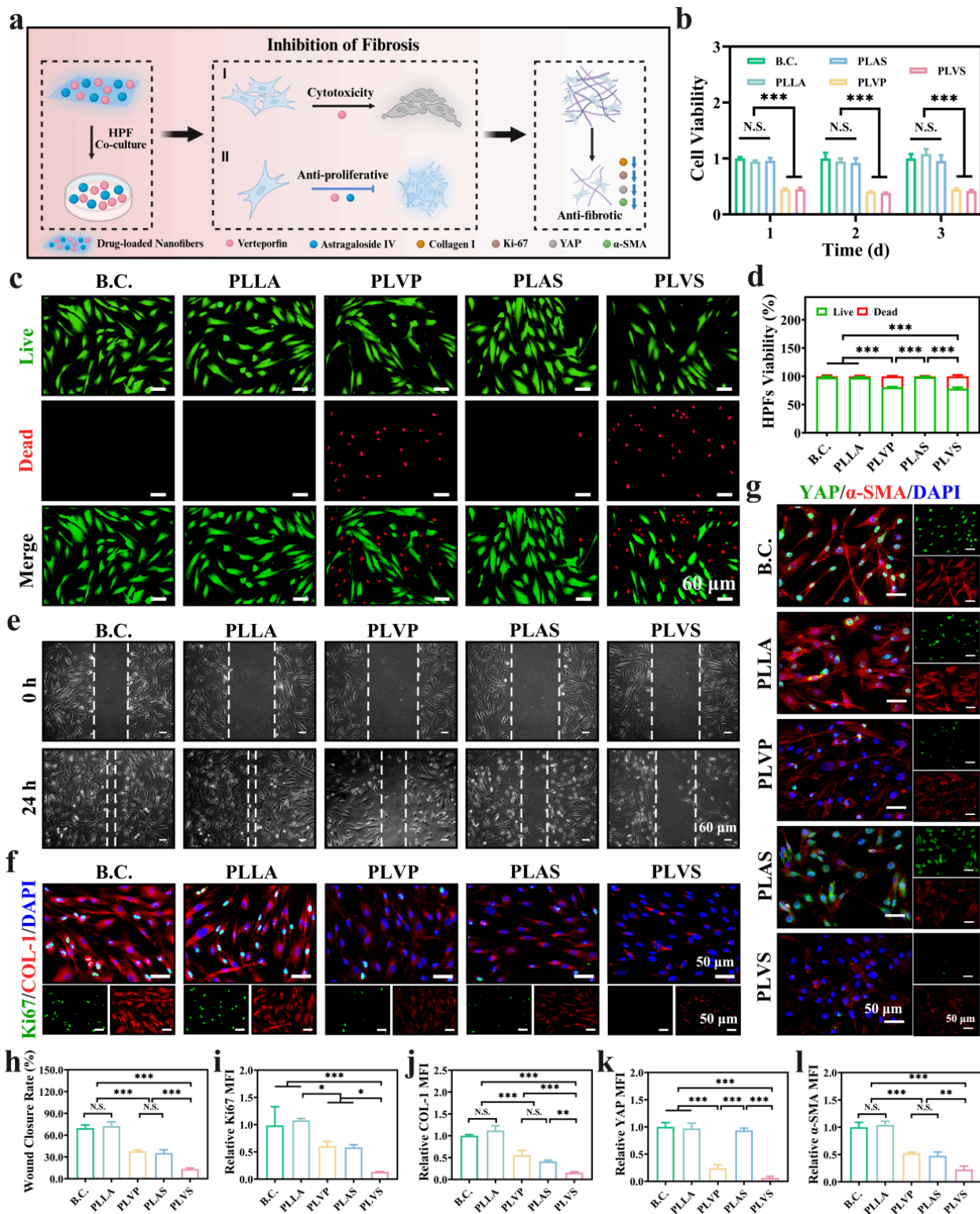


Figure 4

In vitro anti-fibrotic effects of VP/AS co-loaded PLVS nanofibrous membranes via ablating YAP signaling. (a) Schematic overview of the experimental design for exploring the fibroblast-suppressive and anti-fibrotic mechanisms of drug-laden nanofibers, including VP-induced cytotoxicity, anti-proliferative activity and subsequent repression of fibrotic markers. (b) CCK-8 quantitative curves showing HPF viability across blank control (B.C.), PLLA, PLAS, PLVP and PLVS groups at day 1, 2 and 3. (c) Representative

Calcein-AM/PI live/dead fluorescence staining images of HPFs after different treatments, scale bar = 60 μm . (d) Statistical quantification of live and dead HPF proportions calculated from live/dead staining results. (e) Bright-field scratch wound healing micrographs recorded at 0 h and 24 h to assess fibroblast migratory capacity, scale bar = 160 μm . (f) Immunofluorescence staining for proliferation marker Ki67 (red), collagen matrix marker COL-1 (green) and nuclear counterstain DAPI (blue), scale bar = 50 μm . (g) Immunofluorescence co-staining of profibrotic transcription factor YAP (green), myofibroblast biomarker α -SMA (red) and DAPI (blue), scale bar = 50 μm . (h) Quantitative statistics of relative wound closure rates at 24 h post-scratching. (i,j) Relative mean fluorescence intensity (MFI) analysis of Ki67 and COL-1, respectively. (k,l) Relative MFI quantification of YAP and α -SMA expression levels. N.S., not significant. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

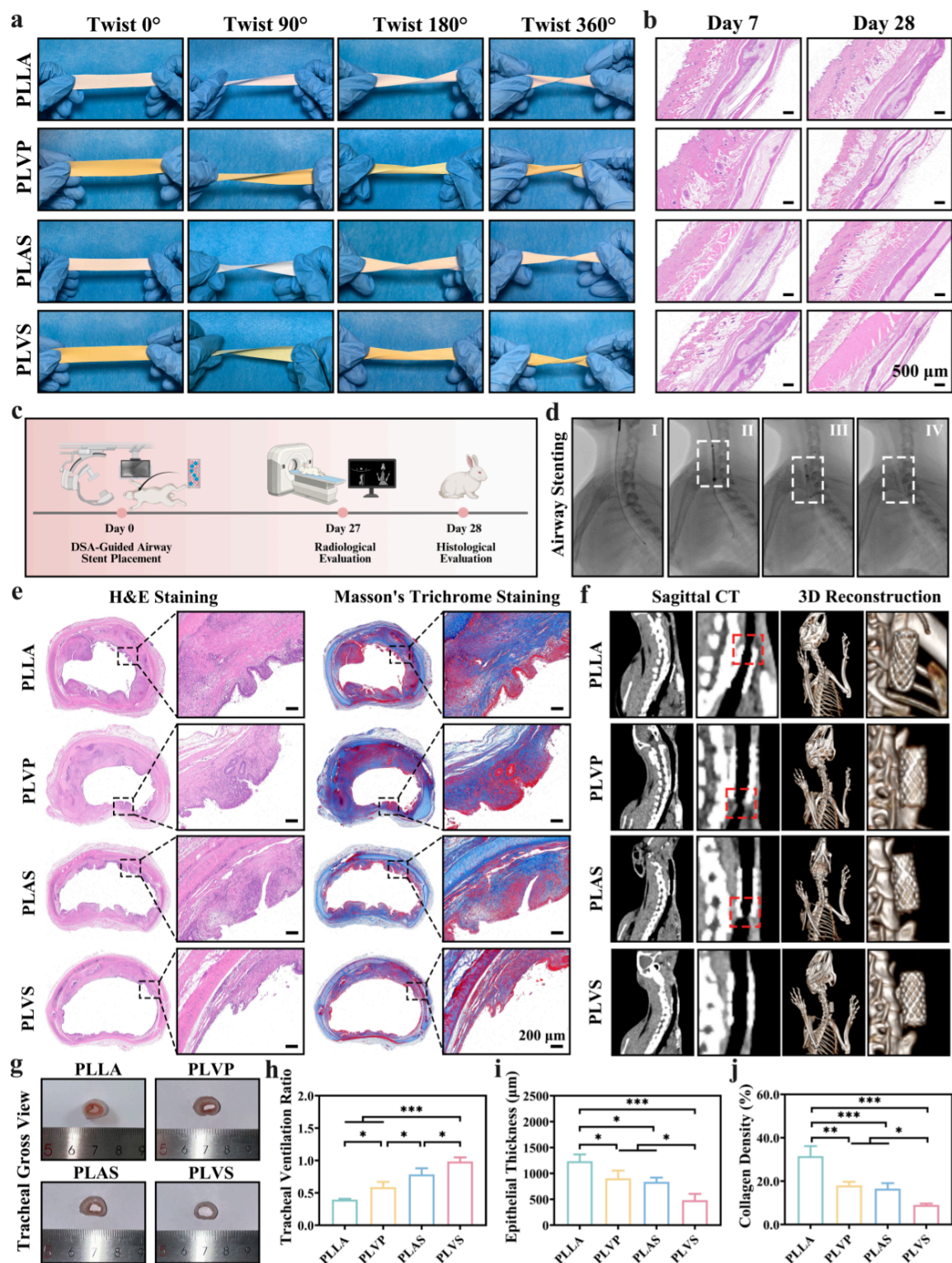


Figure 5

in vivo mechanical adaptability, biosafety and anti-restenosis therapeutic performance of the YAP-antagonistic nanofiber-laminated tracheal stent. (a) Digital photographs showing intact morphology of PLLA, PLVP, PLAS and PLVS nanofibrous membranes after twisting at 0°, 90°, 180° and 360°. (b) H&E histological slices of subcutaneously implanted nanofibrous coatings collected at day 7 and day 28 post-operation to evaluate in vivo degradation and local inflammatory response, scale bar = 500 μm. (c)

Schematic workflow of the rabbit tracheal stenting animal model, including DSA-guided stent deployment at day 0, CT radiological detection at day 27 and terminal histological assessment at day 28. (d) Serial DSA fluoroscopic images documenting precise in situ deployment of four types of tracheal stents (I: PLLA, II: PLVP, III: PLAS, IV: PLVS). (e) Representative H&E and Masson's trichrome staining of transverse tracheal sections harvested 4 weeks after implantation, magnified local pathological regions shown on the right, scale bar = 200 μ m. (f) Sagittal CT tomography and three-dimensional reconstructed tracheal models visualizing intraluminal granulation hyperplasia within stented segments. (g) Gross anatomical photographs of dissected tracheal tissues for intuitive observation of intrastent neotissue overgrowth. (h) Quantitative statistical analysis of tracheal ventilation ratio across all experimental groups. (i,j) Morphometric quantification of epithelial hyperplasia thickness and collagen deposition density from histological slices, respectively. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

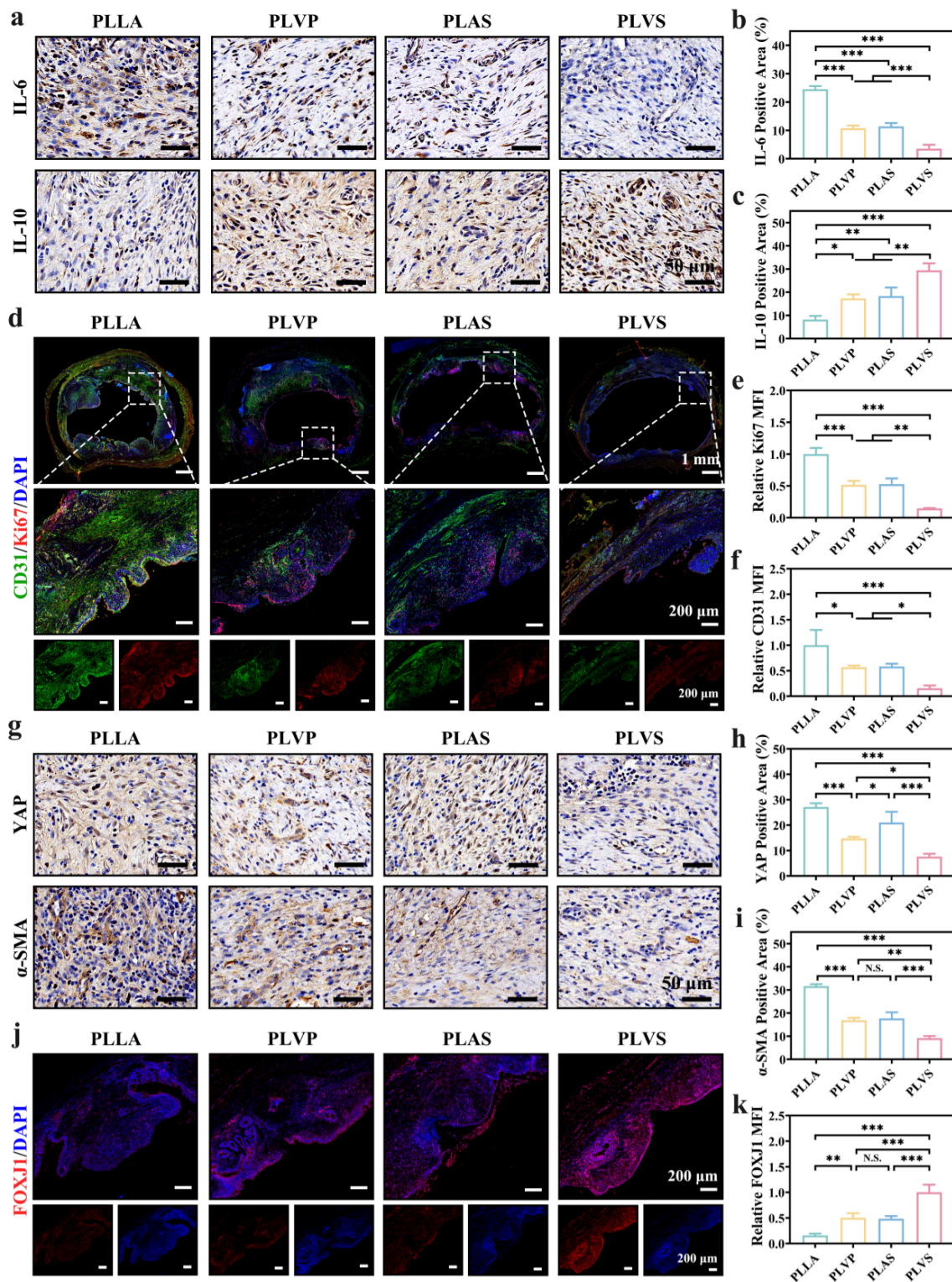


Figure 6

in vivo triple inhibition of inflammation, angiogenesis and YAP-driven fibrosis by the YAP-axis-targeted therapeutic stent coating. (a) Representative immunohistochemical micrographs of pro-inflammatory IL-6 and anti-inflammatory IL-10 in tracheal granulation tissues from all groups, scale bar = 50 μ m. (b,c) Quantitative statistics of IL-6 and IL-10 positive staining area ratios. (d) Multiplex immunofluorescence co-staining for endothelial marker CD31 (green), proliferation marker Ki67 (red) and nuclear DAPI (blue);

Figure 7

Transcriptomic basis of PLVS-suppressed pathological airway remodeling. (a) Volcano plot displaying differentially expressed genes (DEGs) between PLLA control and PLVS treatment groups, with red dots for significantly upregulated genes and blue dots for downregulated genes. (b) Hierarchical clustering heatmap illustrating distinct transcriptional profiles of DEGs across the two cohorts; representative core regulatory genes are labeled. (c,d) GO enrichment bar charts for biological processes enriched in downregulated DEGs, highlighting terms associated with cell adhesion, extracellular matrix assembly and growth factor activity. (e,f) Bubble diagrams of KEGG pathway enrichment analysis for downregulated transcripts, featuring key profibrotic and pro-inflammatory cascades including PI3K-Akt, TGF- β and NF- κ B signaling. (g) GSEA enrichment plots showing suppressed gene signatures linked to inflammatory response, angiogenesis and epithelial-mesenchymal transition (EMT) in PLVS-treated tracheal tissues. (h) Violin plots presenting log₂-transformed transcriptional abundance of hub DEGs (YAP1, MMP1, MMP13, FOXJ1, CXCL5) extracted from RNA-seq datasets. Statistical significance: ***P < 0.001.

Supplementary Files

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- [SupplementaryMaterials.docx](#)
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