

Overcoming Thrombolytic Resistance with a Biomimetic Nanoformulation for Synergistic NETs and Fibrin Degradation in Acute Ischemic Stroke

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Abstract

The efficacy of thrombolysis in acute ischemic stroke with large vessel occlusion (AIS-LVO) is frequently limited by thrombolytic resistance and bleeding risk. The synergy of tenecteplase (TNK) and deoxyribonuclease-I (DNase-I), however, is difficult to translate clinically: DNase-I has a short circulating half-life and low bioavailability and lacks thrombus-targeting capability even when stabilized, while TNK's lack of specificity predisposes to systemic hemorrhage—so a platform that stably, specifically, and synergistically delivers both agents to the thrombus is urgently needed. We therefore engineered a biomimetic nanosystem (TNK-DNaseI@ZPM), co-encapsulating TNK and DNase-I within a platelet membrane-cloaked zeolitic imidazolate framework-8 (ZIF-8) core for precise thrombus targeting and pH-responsive drug release. DNase-I degrades the neutrophil extracellular traps (NETs) scaffold around fibrin, sensitizing thrombi to TNK-driven fibrinolysis. This nanoformulation achieved rapid and superior recanalization while significantly reducing hemorrhagic risk, offering a biomimetic platform to overcome thrombolytic resistance and enable safer, more effective AIS-LVO recanalization.

Background

Acute ischemic stroke with large vessel occlusion (AIS-LVO) is a severe cerebrovascular event associated with mortality and long-term disability rates more than twice those of non-large vessel occlusion stroke^[1]. The abrupt occlusion of intracranial large vessels leads to rapid cerebral infarction, imposing a substantial burden on patients, healthcare systems, and society^[2]. Early recanalization is essential in the acute-phase treatment to salvage the ischemic penumbra and mitigate brain injury^[3]. Although mechanical thrombectomy achieves higher recanalization rates than intravenous thrombolysis for AIS-LVO, its widespread clinical use is constrained by stringent demands for specialized equipment, technical expertise, high costs, and inherent invasive risks.^[4] Intravenous thrombolysis, a standard therapy, mainly uses intravenous recombinant tissue plasminogen activators (e.g., alteplase) to dissolve thrombi, but its efficacy is severely affected by narrow therapeutic windows, poor thrombus specificity, and bleeding risks^[5, 6]. Tenecteplase (TNK), a new-generation intravenous thrombolytic, shows promise in AIS treatment due to superior pharmacology (higher fibrin specificity, longer plasma half-life)^[7]. However, clinical trials indicate that its early recanalization efficacy remains suboptimal^[8].

Traditional views hold that AIS thrombi are mainly composed of fibrin, platelets, and erythrocyte^[9, 10]. Recent studies have revealed that despite high heterogeneity in thrombus components, they share a common structural feature: a shell structure consisting of fibrin, aggregated platelets, von Willebrand factor, etc. Due to the diversity of thrombus components and the core-shell structural characteristics, the efficacy of thrombolytic drugs is directly affected^[11, 12]. Neutrophil extracellular traps (NETs) are extracellular meshwork structures released by neutrophils via "NETosis", composed of DNA, histones, and granular proteins, which can trap and kill pathogens. Under pathological conditions, they participate in thrombosis and inflammatory responses^[13, 14]. Notably, NETs exist in almost all AIS thrombi, acting as a "biological glue". Their DNA skeletons and histones cross-link fibrin, erythrocyte, and platelets to form a

three-dimensional mesh scaffold, which hinders drug penetration by stabilizing the physical structure of thrombi^[15]. Additionally, NETs interact with platelets, promoting coagulation cascades, enhancing platelet aggregation and procoagulant activity, and facilitating platelet adhesion and thrombosis^[16, 17]. NETs have been confirmed to be involved in thrombolysis resistance^[18]. Deoxyribonuclease-I (DNase-I) degrades NETs by recognizing and cleaving DNA backbones^[19], with proven therapeutic potential. Its combination with alteplase significantly enhances thrombolysis^[20, 21]. Our preliminary data show better efficacy of TNK plus DNase-I than TNK alone^[22]. However, translating this synergistic mechanism into clinical practice faces formidable obstacles: DNase-I has a short circulating half-life and low bioavailability, making its in vivo activity difficult to guarantee^[23, 24]; even if its enzymatic activity could be preserved in the systemic circulation, free DNase-I lacks thrombus-targeting capability. Meanwhile, TNK itself carries a risk of systemic hemorrhage due to its lack of specificity. Therefore, there is an urgent need for a platform capable of stably, specifically, and synergistically delivering both drugs to the thrombus.

Nano drug delivery systems (NDDS) offer a promising strategy to overcome the aforementioned challenges. Targeting the thrombus-forming microenvironment, we selected zeolitic imidazolate framework-8 (ZIF-8) as the core nanocarrier. ZIF-8 possesses high drug-loading capacity and favorable biocompatibility^[25]; more importantly, it undergoes pH-responsive dissociation under acidic conditions^[26, 27]. Inspired by this, we constructed a platelet membrane-coated biomimetic NDDS, TNK-DNase-I@ZPM. The platelet membrane endows the nanoparticles with intrinsic thrombus-targeting capability, enabling active accumulation at the thrombus site; the ZIF-8 enables efficient co-encapsulation of TNK and DNase-I and responsive drug release under the acidic thrombus microenvironment (Fig. 1). The results demonstrate that this biomimetic nanosystem achieves stable and specific enrichment at the thrombus site, prolongs in vivo circulation time, significantly enhances thrombolytic efficacy in vivo, and reduces hemorrhagic complications. This study provides a biomimetic nanoplatform with substantial translational prospects for overcoming thrombolysis resistance and achieving safer and more efficient recanalization therapy for AIS-LVO.

Materials and methods

TNK was obtained from CSPC Recogen Pharmaceutical Co., Ltd (Guangzhou, China). DNase-I was obtained from Roche (Switzerland). Zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was acquired from Saen Chemical Technology Co., Ltd (Shanghai, China). 2-MIM was synthesized by Tokyo Chemical Industry Co., Ltd. Polyvinylpyrrolidone (PVP, wt 10000) was obtained from Aladdin Biochemical Technology Co., Ltd (Shanghai, China).

Isolation of platelets and membrane derivation

C57BL/6J mice (8-week-old, male) were anesthetized via intraperitoneal injection of sodium pentobarbital (1%). Whole blood was collected into ethylenediaminetetraacetic acid (EDTA)-

anticoagulated tubes and centrifuged ($100 \times g$, 20 minutes) to separate red and white blood cells. Purified platelet-rich plasma (PRP) was supplemented with PBS containing EDTA (1 mM) and prostaglandin E1 (PGE1, 2 μ M) to prevent platelet activation, followed by centrifugation ($800 \times g$, 20 minutes, room temperature). The pelleted platelets were resuspended in PBS with EDTA (1 mM) and protease inhibitor cocktail, aliquoted into microcentrifuge tubes (1.5 mL), and subjected to three freeze-thaw cycles (-80°C). Lysates were centrifuged ($4,000 \times g$, 3 minutes), and the pellets were washed with PBS/protease inhibitors. The final PM pellets were suspended in ultrapure water and stored at -80°C .

Peripheral blood was collected from healthy volunteers who provided informed consent. Eligibility criteria included: no history of stroke, hematological disorders, or other major conditions affecting coagulation; non-smoking and alcohol-abstinent status; and no use of coagulation-affecting medications within the preceding 3 months (verified by questionnaire). Human PM were extracted using the identical protocol described for mouse platelets.

Preparation and characterization of nanoassemblies

A mixture containing TNK and DNase-I (3 mg TNK and 1 mg DNase-I) was combined with an aqueous solution of 2-MIM (2.5 mM, 1 mL), followed by addition of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (5 M, 0.1 mL) and PVP (3 mg) were added. The reaction proceeded under stirring (20 minutes, room temperature). The product was isolated by centrifugation (10000 rpm, 10 minutes), washed with Milli-Q water (3 $\times$) to remove residual reactants, and vacuum-dried to yield TNK-DNase-I@ZIF-8. TNK@ZIF-8 was prepared identically using TNK (3 mg) and the aqueous solution of 2-MIM as described above.

For membrane coating, the PM solution (1 mg mL^{-1}) was mixed with an equivalent volume of TNK-DNase-I@ZIF-8 for 30 minutes. The mixture was extruded sequentially through polycarbonate porous membranes (800, 400, and 200 nm) using an Avanti Mini-Extruder, followed by centrifugation ($10000 \times g$, 5 minutes) for separation. The resulting coated complex was resuspended in ultrapure water for further use.

The size and zeta potential of nanoassemblies were determined using dynamic light scattering (DLS, Malvern Zetasizer Nano ZS, UK). Morphology was observed by transmission electron microscope (TEM, 80 V, Hitachi H-7650, Japan) after staining with lead citrate (2.6%). The UV spectra (Shimadzu UV-2550, Japan) and FTIR spectra (VERTEX 70, Germany) were measured to evaluate the synthesis of TNK@ZIF-8.

Drug loading capacity and encapsulation efficiency

The DLC and EE are crucial parameters in drug delivery systems, indicating the amount of drug that can be incorporated into a carrier. Generally, DLC and EE percentages are calculated using equations (Eqs. 1 and 2).

DLC refers to the maximum amount of drug that can be loaded onto a carrier or delivery system. It represents the percentage of the total weight of the carrier and drug that is occupied by the carrier

$$DLC\% = \frac{\text{amount of TNK or DNase} - \text{loaded}}{\text{total amount of TNK@ZIF-8 or DNase} - \text{loaded}} \times 100\%$$

1

EE is a measure of how efficiently the drug is loaded onto the carrier, indicating the percentage of the drug successfully incorporated. It represents the percentage of the initially added drug that is effectively loaded onto the carrier.

EE(%) = amount of TNK or DNase- loaded in ZIF-8 amount of cur initially used×100

$$EE\% = \frac{\text{amount of TNK or DNase} - \text{loaded in ZIF-8}}{\text{total amount of TNK or DNase} - \text{initially used}} \times 100\%$$

2

pH-responsive release and stability

TNK@ZIF-8 (5 mg mL⁻¹) was resuspended in PBS at different pH (pH 7.4 and 6.4). Keep the solution at 37°C under slightly shaking. At designated timepoints, the solution was collected by the centrifugation to analyze the released TNK. The release rate of TNK was determined by BCA Protein Test Kit according to the protocol manual.

To evaluate the long-term stability of TNK-DNase@ZIF-8 and TNK-DNase@ZPM were explored by determining the particle sizes of nanoassemblies incubated in PBS (pH 7.4) for one week.

Western blot

Western blot

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to evaluate key membrane proteins in PM and TNK-DNase@ZPM. Samples were prepared at uniform protein concentrations in SDS loading buffer. All samples were heated at 70°C for 10 minutes in a water bath before being loaded onto polyacrylamide gels for separation. Separated proteins were subsequently transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% skim milk for 1 h, and incubated overnight at 4°C with primary antibodies: anti-CD41 (1:1000, Abcam, ab134131), anti-CD61 (1:1000, Proteintech, 18309-1-AP), and anti-CD62p (1:1000, Proteintech, 60322-1-Ig). After washing with Tris-buffered saline containing 0.1% Tween 20 (TBST), membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature for 1

h and washed again with TBST. Protein bands were visualized using a chemiluminescence imaging system (BIO-RAD, USA).

Hemolysis assay

Whole blood was collected from C57BL/6J mice and centrifuged to obtain red blood cells (RBCs). RBCs were resuspended in PBS to prepare a 2% suspension, then incubated with TNK-DNase@ZPM (1 mg mL⁻¹) at room temperature. PBS and ultrapure water served as negative and positive controls, respectively, with (final volume, 2 mL/tube). Samples were photographed hourly. After 3 h, mixtures were centrifuged at 3000 rpm for 3 minutes, and supernatant absorbance at 540 nm was measured using a microplate reader (Thermo Scientific, USA). Hemolysis percentage was calculated as:

$$\text{Hemolysis (\%)} = \left[\frac{(A_{\text{sample}} - A_{\text{neg}})}{(A_{\text{pos}} - A_{\text{neg}})} \right] \times 100\%$$

Samples with > 5% hemolysis were considered hemolytic.

Platelet aggregation assay

Whole blood collected from C57BL/6J mice was anticoagulated with 10% trisodium citrate solution and centrifuged at 850 ×g for 20 minutes to obtain PRP. PRP (1 mL) was transferred to microplate wells and incubated with TNK-DNase@ZPM (1 mg mL⁻¹), thrombin (0.2 U in PBS, positive control), or PBS alone (negative control). Absorbance at 650 nm was continuously monitored using a microplate reader (Thermo Scientific, USA) for at least 20 minutes, with initial values normalized to 100%.

In vitro cytotoxicity

Human umbilical vein endothelial cells (HUVECs) were cultured in 1% Dulbecco's modified eagle medium (DMEM, Gibco, USA) containing 10% fetal bovine serum and penicillin/streptomycin at 37°C in a humidified 5% CO₂. Cells were seeded in 96-well plates at 5000 cells in each well for 12 h, then incubated with TNK-DNase@ZIF-8 at varying concentrations (0-100 µg mL⁻¹) at 37°C. After 24 h incubation, cells were rinsed three times with PBS (0.01 M, pH 7.4) and incubated with fresh medium containing CCK-8 solution (10%, Dojindo, Japan) for 1–2 h. Absorbance at 450 nm was measured using a microplate reader (Thermo Scientific, USA). The DMEM-treated group served as negative control, with cell viability normalized to 100%.

Artificial leukocyte- rich and platelet-rich clots

Peripheral blood from healthy volunteers meeting identical exclusion criteria as for PM isolation was collected into 3.2% sodium citrate anticoagulant tubes. Anhydrous CaCl₂ (16.6 mM) was added to blood

(250 μ L) and incubated at 37°C for 30 minutes to form clot cores. The remaining blood was centrifuged at 120 $\times$ g for 15 minutes to obtain leukocyte-rich and platelet-rich plasma, into which the clot core was placed. The mixture was uniformly incubated in a thermomixer (500 rpm, 37°C) for 3.5 h to form complete clots.

In vitro thrombolysis assay

Fresh thrombi retrieved from AIS-LVO patients undergoing endovascular thrombectomy (EVT) at the First Affiliated Hospital of Jinan University (November 2024–January 2025) were analyzed in vitro (ethical approval KY-2024-223). Thrombi were incubated in 6-well plates at 37°C for 1 h with the following treatments: PBS, TNK (1 μ g mL⁻¹), TNK (1 μ g mL⁻¹)+DNase-I (100 IU mL⁻¹), TNK@ZIF-8 (1 μ g mL⁻¹ TNK, pH 6.4/7.4), TNK-DNaseI@ZIF-8 (1 μ g mL⁻¹ TNK, pH 6.4/7.4), or TNK-DNaseI@ZPM (1 μ g mL⁻¹ TNK, pH 6.4/7.4), all containing supplemental plasminogen (1 μ M). Thrombus weights were recorded at 0, 20, 40 and 60 minutes by ultra-microbalance. Thrombolysis efficacy expressed as percentage residual mass relative to initial weight where lower values indicated enhanced lysis. Identical protocols were applied to artificial blood clots.

Animal studies

Eight-week-old male C57BL/6J mice (23–26 g) were obtained from Bestest Biological Technology Co., Ltd (Zhuhai, China). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Jinan University (IACUC-20240923-09) and conducted in accordance with the ARRIVE guidelines.

Hemorrhage volume assessment

To quantify TNK-associated hemorrhage risk and determine the optimal dose for TNK-DNaseI@ZPM in vivo, mice subjected to photochemical thromboembolism received tail-vein TNK at 2.5, 5.0, 7.5, or 10.0 mg kg⁻¹ 2 h post-induction; 24 h later, brains were perfused with saline and hemorrhage volume was calculated as percentage of total brain volume using Image-J.

FeCl₃-induced carotid artery thrombosis model

After intraperitoneal pentobarbital (1%) anesthesia, a 1 cm midline cervical incision exposed the left common carotid artery, which was bluntly dissected and wrapped for 10 minutes with 10% FeCl₃-soaked filter paper; the vessel was then rinsed with sterile PBS. Thrombosis was confirmed by laser speckle contrast imaging (LSCI, Moore FLPI-2) when baseline flow fell $\geq$ 70%. Mice received tail-vein saline, TNK (2.5 mg kg⁻¹), TNK (2.5 mg kg⁻¹) + DNase-I (50 U), or equivalent-TNK TNK@ZIF-8, TNK-DNaseI@ZIF-8, or TNK-DNaseI@ZPM. Reperfusion was monitored for 20 minutes, then the occluded segment was excised, HE-stained, and thrombus area relative to total luminal cross-section was quantified using Image-J.

Photothrombotic stroke model

Following anesthesia, mice received tail-vein rose bengal (50 mg kg⁻¹, Sigma-Aldrich) and, 5 minutes later, a 532 nm fiber-optic beam (5 mW) was focused for 16 minutes 30 seconds on a 2 mm burr hole

over the left middle cerebral artery; stable thrombosis was defined as $\geq 70\%$ flow reduction by LSCI. Two hours later, saline, TNK (2.5 mg kg^{-1}), TNK (2.5 mg kg^{-1}) + DNase-I (50 U), or equivalent-TNK TNK@ZIF-8, TNK-DNaseI@ZIF-8, or TNK-DNaseI@ZPM were administered intravenously. Cerebral perfusion was serially recorded pre-modeling and at 20, 40, and 60 minutes post-injection, with data expressed as percentage of baseline flow.

Twenty-four hours after modeling, neurological deficits were graded by the Longa scale: 0 = no deficit, 1 = incomplete right fore-or hind-limb extension, 2 = circling to the right, 3 = falling to the right, 4 = inability to walk spontaneously with reduced consciousness. Brains were removed, chilled at -20°C for 30 minutes, cut into 2 mm coronal slices, incubated in triphenyltetrazole chloride (TTC, 2%, Sigma-Aldrich, 37°C , 30 minutes, inverted once at 15 minutes), fixed in paraformaldehyde (PFA, 4%, Sigma-Aldrich), and analyzed with Image-J to determine infarct volume as a percentage of total brain volume.

Immunofluorescence

PTS mouse brains were fixed in PFA (4%), paraffin-embedded longitudinally, deparaffinized, and antigen-retrieved in Tris-EDTA (pH 6.0, Dako). After PBS washes (3×5 minutes), sections were blocked with bovine serum albumin (BSA, 5%, Sigma-Aldrich) for 30 minutes at room temperature, incubated overnight at 4°C with rabbit anti-citrullinated histone H3 (1:500, Abcam, ab5103) and mouse anti-myeloperoxidase (1:500, Abcam, ab25989), followed by Alexa Fluor 488 anti-rabbit (1:300, ServiceBio, GB21303) and Cy3 anti-mouse (1:400, ServiceBio, GB25301) for 1 hour at room temperature. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). Images were acquired on a Zeiss LSM710 confocal microscope; NETs were defined by extracellular DNA co-localized with citrullinated histone H3 (H3-Cit) and myeloperoxidase (MPO).

Nanoparticle targeting and biodistribution

Indocyanine green (ICG)-labeled TNK-DNaseI@ZIF-8 or TNK-DNaseI@ZPM (2.5 mg kg^{-1} TNK) was administered intravenously to mice with FeCl_3 -induced carotid thrombosis or PTS mice. Near-Infrared II (NIR-II) whole-body imaging (DPM-IVFM-NIR-II, Dipu Medical, Zhuhai) tracked fluorescence location and intensity for 2 hours, expressed as signal-to-background ratio (SBR). After euthanasia, heart, liver, spleen, lung, and kidney were harvested to map organ distribution.

Pharmacokinetics

To evaluate nanoparticle pharmacokinetics, ICG-labeled TNK-DNaseI@ZIF-8 or TNK-DNaseI@ZPM (1 mg mL^{-1}) was injected intravenously into naive mice; orbital blood was collected at 1, 10, 30 minutes and 1, 4, 8, 24, 48 hours post-dose. Fluorescence intensity in blood was measured by NIR-II imaging; the 1-minutes signal was set as 100%, and subsequent values are expressed as percentages of this baseline.

Safety assessment

Seven days after tail-vein administration of saline, TNK, or TNK-DNase-I@ZPM (2.5 mg kg^{-1} TNK) to PTS mice, whole blood was collected for biochemical (ALT, AST, UREA, CREA, UA) and coagulation (PT, APTT,

TT, FIB) assays; heart, liver, spleen, lung, and kidney were harvested for HE.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). Comparisons among groups used one-way ANOVA with Tukey's post-hoc (normal, equal variance) or Kruskal–Wallis test otherwise. Repeated-measure outcomes (in-vitro thrombolysis, PTS CBF, nanoparticle targeting) were analyzed by two-way repeated-measures ANOVA. All analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA); $P < 0.05$ was considered statistically significant.

Results and Discussion

Characterization of TNK-DNase-I@ZPM

The nanoparticle preparation process was detailed. Dynamic light scattering (DLS) revealed that pristine ZIF-8 exhibited an average hydrodynamic diameter of 114.05 ± 7.8 nm; After co-encapsulation of TNK and DNase-I, the particle size increased to 155.35 ± 28.94 nm, forming TNK-DNase-I@ZIF-8 (TD@Z). Subsequent PM coating further increased the hydrodynamic diameter to 196.20 ± 32.74 nm for TNK-DNase-I@ZPM (TD@ZPM) (Fig. 2D), indicating successful stepwise construction of the core–shell structure. Consistent with drug loading and membrane coating, the surface charge of the nanoparticles underwent a marked transition. Pristine ZIF-8 displayed a positive zeta potential of $+16.80 \pm 1.05$ mV, which decreased to -14.70 ± 0.82 mV after loading negatively charged TNK and DNase-I. Following PM coating, TD@ZPM exhibited a further reduced zeta potential of -19.63 ± 1.16 mV (Fig. 2E), mainly due to the charge screening effect after the membrane coating.

Transmission electron microscopy (TEM) showed that TNK@ZIF-8 (T@Z) (**Supporting Information, Figure S1**) and TD@Z (Fig. 2B) retained the typical rhombic dodecahedron morphology of ZIF-8 (Fig. 2A). In contrast, TD@ZPM (Fig. 2C) shows a slightly elliptical morphology with a distinct outer membrane layer while maintaining a clear crystal core. These observations directly verify the effective integration of PM and indicate that neither protein loading nor membrane coating compromised the structural integrity of the ZIF-8. The successful encapsulation of TNK was further confirmed by spectroscopic analyses. Ultraviolet-visible (UV-Vis) spectra of T@Z exhibited both the characteristic absorption peak of TNK and the scattering signal associated with ZIF-8 (Fig. 2G). Fourier transform infrared (FTIR) spectra showed the coexistence of the imidazole ring vibration of ZIF-8 and the amide bond signals of TNK (Fig. 2H), collectively confirming the formation of the nanoparticle.

The colloidal stability of the nanoparticles was evaluated by incubating TD@Z and TD@ZPM in phosphate-buffered saline (PBS, pH 7.4). TD@ZPM maintained a stable hydrodynamic size over a period of one week, whereas TD@Z exhibited pronounced particle aggregation (**Supporting Information, Figure S 2A-B**). This result clearly reflects the advantage of the cell membrane coating. PM can simulate the surface properties of natural cells, reduce non-specific aggregation of nanoparticles in physiological environments, and improve the stability^[31]. The core value of PM lies in its surface being rich in

thrombus-targeting related proteins^[32]. To examine whether key platelet-derived proteins were retained after membrane coating, Western blot analysis was performed. TD@ZPM preserved representative platelet membrane markers, including CD41, CD61, and CD62p (Fig. 2F), confirming the successful translocation of functional membrane proteins onto the nanoparticle surface.

The drug release behavior is a core property that enables nanoparticles to protect drugs during circulation and release them at target sites^[33]. The pH-responsive release behavior of TNK from ZIF-8 was subsequently investigated. Under physiological conditions (pH 7.4), the cumulative release of TNK from T@Z reached only 23.45% within 24 h. In contrast, under mildly acidic conditions (pH 6.4), which mimic the microenvironment of thrombus, TNK release increased dramatically to 98.88% over the same period (Fig. 2I), demonstrating a pronounced pH-triggered release profile. The obvious pH-responsive release is highly beneficial for the targeted therapy of the thrombus. Nanoparticles can minimize the leakage of drug in the blood (pH 7.4) and rapidly release the loaded drug at the thrombus site, ensuring a high effective therapeutic concentration at the target location while reducing systemic toxicity.

Finally, the encapsulation efficiency (EE) and drug loading capacity (DLC) of ZIF-8 for proteins were quantified. TNK exhibited an EE and DLC of 73.19% and 10.40%, respectively, while DNase-I showed an EE of 82.40% and a DLC of 1.65% (**Supporting Information, Table S1**), indicating the high loading capability of ZIF-8 toward protein-based drugs.

Biocompatibility and cytotoxicity of TNK-DNase α @ZPM

Biocompatibility is a core prerequisite for the in vivo application of nanoparticles^[34]. Hemolysis assays were conducted to evaluate the safety of TD@ZPM toward erythrocytes, as erythrocyte membrane rupture may trigger adverse immune responses or vascular complications^[35]. The centrifuge tube of the positive control group (ddH₂O) showed a large amount of red supernatant (indicating erythrocyte rupture), while the supernatants of the PBS group and TD@ZPM group remained clear, with only erythrocyte precipitation at the bottom of the tube (**Supporting Information, Figure S 3A**). Quantitative results (**Supporting Information, Figure S 3B**) further showed that the hemolysis rate of the TD@ZPM group was less than 1%, which is far below the safety threshold of biomaterials (usually 5%). The result indicates that TD@ZPM does not damage the integrity of erythrocyte membranes and has good blood compatibility. Abnormal platelet activation may induce thrombosis^[36], so the anti-platelet activation property of nanoparticles is an important guarantee for their circulatory safety. Platelet aggregation assay (**Supporting Information, Figure S3 C**) showed that the relative turbidity of the PBS group remained stable (close to 100%) within 20 minutes, and that of the TD@ZPM group also exhibited no significant change; while the relative turbidity of the positive control group (thrombin) decreased significantly after 15 minutes (indicating massive platelet aggregation). This result suggests that TD@ZPM does not activate platelets and has no risk of prothrombotic during circulation. Endothelial cells are important components of blood vessels, and their activity directly affects vascular function^[37]. ZIF-8 exhibited minimal cytotoxicity at concentrations below 100 $\mu\text{g mL}^{-1}$ (**Supporting Information,**

Figure S 3D), while TD@Z did not reduce endothelial cell viability across a range of concentration gradients (**Supporting Information, Figure S 3E**). This result verifies the low toxicity of TD@Z to endothelial cells, providing support for the tissue safety of its in vivo application.

Combined with the above results, TD@ZPM has no hemolysis risk in the blood environment, does not induce platelet activation, and has no obvious toxicity to endothelial cells. These characteristics together prove that it has good biocompatibility, laying a safe foundation for subsequent in vivo thrombolytic applications.

In vitro thrombolysis of TNK-DNase-I@ZPM

We recorded the dissolution of thrombi obtained from AIS-LVO patients within 60 minutes in vitro experiment. As shown in Fig. 3A, thrombi in the PBS group showed no significant dissolution throughout the observation period, with a clear thrombus mass remaining at the bottom. Both the TNK and TNK+DNase-I groups exhibited thrombolytic effects at 60 minutes. The combination group demonstrating superior efficacy, with more fragmented residual mass at the bottom. The quantitative residual thrombus weight results in Fig. 3C further supported the visual observation, reflected by a lower residual thrombus weight (TNK+DNase-I vs TNK: 31.17% vs 46.63%, $P < 0.001$). The core of this difference is the synergistic effect of DNase-I. AIS-LVO thrombi are usually rich in NETs, which enhance thrombus stability by interweaving fibrin via DNA skeletons. DNase-I can specifically degrade the DNA structure of NETs, removing its "physical barrier" to TNK, thus amplifying the fibrinolytic effect^[12, 38]. For nano-drug-loaded formulations, the thrombolytic performance varied significantly under different pH environments. At pH 7.4, the T@Z group had a residual thrombus weight of 92.62%, which directly correlates with ZIF-8's stable structure and slow drug release in the neutral environment. The TD@Z group showed 87.25% residual weight, slightly better than T@Z but still limited in overall effect, while the residual weight of the TD@ZPM decreased to 68.73%, significantly better than the previous two groups (TD@Z vs T@Z, $P < 0.0001$; TD@ZPM vs TD@Z, $P < 0.0001$, **Supporting Information, Figure S 4A**). This advantage stems from the thrombus-targeting effect of platelet membrane. The adhesion proteins on the PM surface mediate nanoparticle enrichment at the thrombus site, increasing local drug concentration and partially compensating for insufficient drug release in neutral environments. At pH 6.4, thrombolytic efficiency was further enhanced across all drug-loaded formulations. The T@Z and TD@Z groups exhibited residual weights of 76.17% and 70.08%, and that of the TD@ZPM group only to 27.28%. This result reflects both the pH responsiveness of ZIF-8, the synergistic effect of PM-mediated targeting and combined therapy. Notably, the thrombolytic effect of the TD@ZPM group was not statistically different from that of the TNK+DNase-I group (TD@ZPM vs TNK+DNase-I, $P = 0.13$), but significantly better than all other drug-loaded groups (TD@ZPM vs other groups, $P < 0.0001$). Although the initial thrombolytic rate of TD@ZPM lagged behind that of the TNK+DNase-I group, likely due to the delayed drug release from the ZIF-8 framework. However, there is no significant difference in thrombolysis efficiency was observed between the two groups at 60 minutes. Notably, approximately about 30% of the total drug payload was released during this period, yet the thrombolytic effect was pronounced, underscoring the high efficiency of TD@ZPM. Targeting enrichment makes the local effective drug

concentration at the thrombus far higher than the systemic distribution concentration of free drugs, thus achieving comparable thrombolytic effect with lower drug release.

To further validate these findings and mitigate the confounding effects of compositional heterogeneity in clinical thrombus samples^[10, 39], we constructed artificial leukocyte- rich and platelet-rich clots using peripheral blood from healthy volunteers and repeated the thrombolysis experiments to verify result reliability. Results were consistent with the trends observed in AIS-LVO thrombi (Fig. 3B). No dissolution occurred in the PBS group. The TNK group showed a residual clot weight of 51.38% at 60 minutes, while the TNK+DNase-I group exhibited a significant reduction to 31.10% (TNK+DNase-I vs TNK, $P < 0.01$), reaffirming the synergistic thrombolytic effect of DNase-I^[21]. At pH 7.4, the residual rates for T@Z, TD@Z, and TD@ZPM were 92.43%, 85.50%, and 66.30% (TD@Z vs T@Z, $P < 0.01$; TD@ZPM vs TD@Z, $P < 0.001$, At pH 6.4, thrombolytic efficiency improved further across all nanoparticle groups, with residual rates of 77.93% for T@Z, 70.52% for TD@Z, and 28.22% for TD@ZPM (TD@Z vs T@Z, $P < 0.05$; TD@ZPM vs TD@Z, $P < 0.0001$, Fig. 3D, **Supporting Information, Figure S 4B**). Importantly, TD@ZPM at pH 6.4 demonstrated significantly greater thrombolytic efficacy than the TNK+DNase-I group ($P < 0.05$), this result further strengthens the advantage of TD@ZPM.

Assessment of hemorrhagic risk

Hemorrhage is one of the most critical safety hazards in the clinical application of thrombolytic drugs. Firstly, we evaluated the dose-dependent hemorrhagic risk of free TNK, then verified the improvement of hemorrhagic risk by the TD@ZPM. In evaluating the hemorrhagic risk of TNK, we observed an 80% mortality rate in mice within 24 hours following administration of 10.0 mg kg⁻¹, indicating that the systemic fibrinolytic activation of the drug at this dose was too strong, with the poor safety. Therefore, this dosage was excluded from subsequent experiments. As the TNK concentration decreased from 7.5 mg kg⁻¹ to 2.5 mg kg⁻¹, a corresponding reduction in cerebral hemorrhage volume was observed (5.0 mg kg⁻¹ vs 7.5 mg kg⁻¹: 10.39% vs 14.84%, $P < 0.0001$; 2.5 mg kg⁻¹ vs 5.0 mg kg⁻¹: 8.63% vs 10.39%, $P < 0.05$) (**Supporting Information, Figure S5**). This result clarifies that the hemorrhagic risk of free TNK is dose-dependent. As the drug dose decreases, systemic non-targeted fibrinolytic activation weakens, and the hemorrhagic risk decreases accordingly^[40]. Based on these results, the 2.5 mg kg⁻¹ concentration was selected for subsequent in vivo thrombolysis assays. Further administration of TD@ZPM (containing TNK 2.5 mg kg⁻¹) in PTS model mice resulted in a significantly smaller hemorrhage volume compared to free TNK at the same concentration (TD@ZPM vs TNK: 5.58% vs 8.63%, $P < 0.01$). TD@ZPM enriches at the thrombus site through the targeting effect of platelet membrane, and releases drugs only in the acidic microenvironment of the thrombus, which greatly reduces the exposure of free drugs in circulation, thus avoiding interference with the fibrinolytic system of normal blood vessels. This result proves that TD@ZPM significantly reduces the hemorrhagic risk while maintaining the thrombolytic dose, improving the in vivo safety of the nanoparticle.

Accumulation of TNK-DNase-I@ZPM at the thrombus site

In the FeCl₃-induced carotid artery thrombosis model, the TD@Z group exhibited only weak, diffuse fluorescence signals in the cervical region at all observation time points (0, 3, 10 to 120 minutes), with no obvious focal enrichment, indicating poor thrombus targeting (Fig. 4A). In contrast, the TD@ZPM group showed clear focal fluorescence in the cervical thrombus area as early as 3 minutes post-injection; the signal remained strong and localized between 10 to 60 minutes, and gradually weakened after 90 minutes (while still confined to the target region), reflecting the ideal targeting behavior of “rapid enrichment, sustained retention, and gradual clearance”. A similar targeting pattern was observed in the PTS model (Fig. 4B). Quantitative results of the Signal-to-Background Ratio (SBR) (Figs. 4E, F) further quantified the differences between the two formulations. Overall, the significantly stronger thrombus-targeting capacity of TD@ZPM over TD@Z across both models (30–120 minutes, $P < 0.0001$) can be attributed to the natural PM coating, which contains key proteins such as CD41, CD47 and CD62p that promote specific binding to activated platelets and injured endothelia^[41]. This finding aligns with recent advances in biomimetic nanomedicine, where cell membrane-coated nanoparticles are increasingly employed for targeted drug delivery in vascular diseases^[42, 43]. The rapid enrichment and sustained retention of TD@ZPM ensure rapid onset and sustained therapeutic concentrations at the target site during acute thrombotic events; meanwhile, the targeting performance gap between TD@Z and TD@ZPM highlights the critical role of biofunctional modification in enhancing the therapeutic efficacy of synthetic nanocarriers.

Biodistribution and pharmacokinetics of TNK-DNase@ZPM

The in vivo distribution and pharmacokinetic behavior of nanoparticles directly determine the therapeutic efficacy and safety. Here, we systematically evaluated these properties of TD@Z and TD@ZPM in animal models. The biodistribution and pharmacokinetic behavior of the nanoparticles were evaluated in two animal models. At 2 hours post-administration, strong fluorescence signals were detected in the liver and kidneys of mice treated with both TD@Z and TD@ZPM, while lower intensities were observed in the spleen, heart, lungs, and brain (Fig. 4C). Quantitative tissue fluorescence intensity results (Figs. 4H-I) further confirmed that hepatic and renal routes were the primary pathways for nanoparticle metabolism. This distribution pattern is consistent with the typical behavior of synthetic nanoparticle^[44, 45], but it also necessitates further investigation into potential off-target toxicity. Although prior cytotoxicity assays demonstrated low TD@Z toxicity to endothelial cells, organ-specific toxicity (e.g., impacts on liver/kidney function) under long-term in vivo exposure requires subsequent validation. This is a critical safety assessment step for the translation of biomimetic nanoparticle from the laboratory to clinical settings^[46].

Blood fluorescence imaging and quantitative analysis revealed distinct pharmacokinetic profiles between the two nanoparticles. TD@Z entered systemic circulation rapidly, with high fluorescence intensity in blood at 1 minute post-injection. However, the signal decreased substantially by 4 hours and became nearly undetectable after 24 hours, indicating rapid clearance. In contrast, TD@ZPM also achieved rapid systemic distribution but exhibited prolonged blood circulation, with measurable

fluorescence maintained from 4 to 24 hours and only minimal signal detectable at 48 hours (Fig. 4D). The extended circulation half-life of TD@ZPM can be attributed to the biomimetic PM coating, which enhances the stability of nanoparticle, and reduces non-specific aggregation in circulation. On the other hand, the CD47 on PM mitigates rapid clearance by the mononuclear phagocyte system^[47]. Notably, this prolonged circulation does not induce premature drug release or additional hemorrhagic risk under physiological conditions, consisting with previous results. The synergistic property of prolonged circulation and targeted enrichment represents the core advantage of TD@ZPM. Prolonged circulation provides a sufficient time window for nanoparticle enrichment at thrombus sites, while targeting ensures concentrated drug release in the target area. This combination effectively expands the therapeutic window and reduces potential toxicity caused by off-target effects.

In vivo thrombolysis of TNK-DNase-I@ZPM

The FeCl₃-induced carotid thrombosis model is a classic in vivo model simulating large-vessel occlusion in ischemic stroke. Having established the thrombus-targeting capability of TD@ZPM, we further evaluated its in vivo thrombolytic efficacy. We evaluated the thrombolytic efficacy of TD@ZPM via laser speckle contrast imaging (LSCI) and Hematoxylin-Eosin (HE) staining. LSCI revealed varying degrees of carotid blood flow recovery across all treatment groups. TD@ZPM restored 77.80% of baseline blood flow in occluded vessels at 20 minutes post-administration, significantly outperforming the 50.51% recovery of the free TNK+DNase-I group ($P < 0.0001$), while other groups (e.g., TNK, TD@Z) failed to meet the clinical threshold for effective recanalization in ischemic stroke ($> 70\%$ of baseline flow) (Fig. 5A). The significantly enhanced reperfusion efficacy of TD@ZPM at the 20-minute time underscores its rapid targeting and drug release capabilities at the thrombus site. This accelerated recovery is critical in ischemic stroke, where early restoration of blood flow is strongly associated with improved clinical outcomes^[48]. Histological analysis via HE staining of the isolated thrombosed carotid arteries showed reduced residual thrombus areas in all treatment groups except the saline control. TD@ZPM restored near-normal vessel lumens with only small amounts of lightly stained thrombus fragments (Fig. 5B), and its residual thrombus area (as a percentage of total vessel area) was far lower than all other groups (TD@ZPM vs TNK+DNase-I, $P < 0.01$; TD@ZPM vs all other groups, $P < 0.0001$). PM-mediated rapid targeting enables drug accumulation at thrombus sites within the golden recanalization window of ischemic stroke (recanalization within this window improves functional prognosis^[49]), while the acidic thrombus microenvironment triggers ZIF-8 dissociation. The released DNase-I degrades NETs, and TNK dissolves fibrin, this dual action is more thorough than single-mechanism formulations. Meanwhile, TD@ZPM's blood flow recovery is closer to the clinical TICI 2b/3 recanalization standard ($> 70\%$ flow recovery)^[50], and its lower thrombus residue reduces reocclusion risk, all of which highlight its translational potential.

To better simulate the clinical scenario of ischemic stroke, we established a PTS model to further evaluate the thrombolytic efficacy of TD@ZPM and investigate NETs expression (Fig. 6A). LSCI revealed differential restoration of cerebral blood flow (CBF) among groups, except for the saline control (Fig. 6B).

At 60 minutes post-administration, the TNK group showed CBF recovery of 31.72% of baseline, while the TNK+DNase-I group achieved significantly higher restoration (61.00%, TNK+DNase-I vs. TNK, $P < 0.0001$), demonstrating synergistic thrombolytic effects. In contrast, T@Z and TD@Z exhibited limited recovery ($< 30\%$ of baseline), likely due to lack of targeting capability and insufficient drug accumulation at the thrombus site. TD@ZPM yielded the most substantial CBF recovery, reaching 72.44% of baseline at 60 minutes (TD@ZPM vs. TNK+DNase-I, $P < 0.001$; TD@ZPM vs other groups, $P < 0.0001$). Although TD@ZPM showed delayed efficacy at 20 minutes (TNK+DNase-I vs TD@ZPM: 37.61% vs 31.90%, $P < 0.01$), it caught up by 40 minutes (TNK+DNase-I vs TD@ZPM: 50.16% vs 49.48%, $P = 0.97$) and surpassed the free drug combination at 60 minutes. This result stems from TD@ZPM's targeted enrichment, which prolongs drug retention at the thrombus site and compensates for the rapid clearance of free drugs.

Neurological function assessed by Longa scores indicated significant improvement in the TD@ZPM group compared to the saline and T@Z groups ($P < 0.01$), though no significant difference was observed relative to other treatment groups (Fig. 6E). Furthermore, infarct volume was reduced across all treatment groups, with the most pronounced reduction observed in the TD@ZPM group (TD@ZPM vs TNK+DNase-I, $P < 0.001$; TD@ZPM vs other groups, $P < 0.0001$, Fig. 6C–F), underscoring its therapeutic potential for ischemic stroke. Notably, while TD@ZPM significantly improved hemodynamic recovery and reduced infarct volume compared to TNK+DNase-I, intergroup neurological scores showed less pronounced differences. This partial mismatch the pathological complexity of ischemic stroke: neurological recovery depends not only on revascularization but also on neuroprotection, inflammation modulation, etc.^[51], highlighting the potential of multimodal nanotherapeutics for comprehensive stroke treatment. Combined with results from the FeCl₃ carotid model, TD@ZPM demonstrates core advantages of efficient recanalization and low deficiency across different thrombus models, and its biomimetic targeting and responsive release design provides a feasible strategy for precision thrombolysis in ischemic stroke.

Expression of NETs in cerebral ischemic regions of PTS mice.

NETs act as dual pathogenic mediators in ischemic stroke. Their DNA skeletons interweave fibrin to enhance thrombus stability, while NETs release exacerbates blood-brain barrier disruption and neuronal apoptosis^[52]. We evaluated NETs expression in ischemic brain regions of PTS mice via immunofluorescence staining (Fig. 7). NETs were detected in the ischemic areas across all treatment groups. Both the TD@ZPM and TNK+DNase-I groups showed reduced NETs expression, with TD@ZPM likely exhibiting superior efficacy, potentially attributable to the NET-degrading effect of DNase-I delivered via the nanoparticle platform. This results in efficient degradation of the NETs scaffold, which compromises thrombus stability and enhances the accessibility of TNK to fibrin strands^[21]. Meanwhile, TD@ZPM may reduce NETs-mediated inflammatory injury, consisting with the minimal infarct volume observed in the TD@ZPM group in the PTS model.

In vivo safety of TNK-DNase α @ZPM

In vivo safety is a core barrier for the clinical translation of nanoparticles, especially for thrombolytic agents, which need to avoid both hepatorenal toxicity and systemic coagulation disorders. We systematically verified the in vivo safety of TD@ZPM via biochemical indices, coagulation function, and histological staining, and compared with saline and TNK groups (**Supporting Information, Figure S 6A**). No significant differences were observed in the function markers of liver and kidney (including ALT, AST, UREA, CREA, and UA) among the three groups, indicating no overt hepatorenal toxicity despite the hepatic and renal clearance suggested by biodistribution studies. This may be attributed to the biomimetic PM coating, which can reduce the non-specific cytotoxicity of nanoparticles in hepatic and renal tissues. Among coagulation parameters (including PT, APTT, TT, and FIB), although TD@ZPM showed a significant difference in APTT shortening compared to TNK ($P < 0.05$), it remained comparable to the saline control. No significant differences were found in other parameters. Free TNK non-specifically dissolves fibrin throughout systemic circulation, disrupting overall coagulation balance. In contrast, TD@ZPM releases drugs only at thrombus sites, significantly reducing disturbance to the systemic coagulation system, and may present a lower bleeding risk than free TNK. Furthermore, HE staining of major organs in PTS model mice revealed normal tissue architecture without apparent damage, inflammatory infiltration, or cytopathological changes in the TD@ZPM group, similar to saline and TNK groups (**Figure S 6B**), its biomimetic PM coating not only enhances targeting but also reduces the immunogenicity and tissue injury risk of nanoparticles^[53], providing histological evidence for its favorable in vivo safety profile.

Conclusion

This study reports a biomimetic nanosystem (TNK-DNase α @ZPM) constructed by co-loading TNK and DNase α into ZIF-8 and coating with natural PM, as a novel thrombolytic strategy for AIS-LVO. The PM confers thrombus-targeting ability, enhancing the precise delivery of ZIF-8 nanocarriers to thrombus sites. The mesoporous structure of ZIF-8 enables efficient encapsulation of both TNK and DNase α , allowing synergistic degradation of fibrin and NETs. The pH-responsive drug release ensures specific payload delivery within the acidic thrombotic microenvironment. This biomimetic nanosystem demonstrates excellent biocompatibility and safety, achieves efficient thrombolysis both in vitro and in vivo, and reduces cerebral hemorrhage volume, offering a targeted, safe, and effective nano therapeutic strategy for AIS-LVO.

Declarations

Supplementary materials

Supplementary figures and tables.

Data availability

Data will be made available on request.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Authors' contributions

Shuhan Xu and Caiqi Yang designed and performed the experiments, prepared the figures, and analyzed the experimental data. Shuhan Xu wrote the original draft of the manuscript. Xia Yang, Liang Zhang, Min Guan, Yu Liao, and Xiaomei Xie collected the clinical thrombus samples and contributed to data analysis. Zhen Jing performed data analysis and verified the integrity and accuracy of the data. Jian Dai and Li'an Huang supervised the project, reviewed and revised the manuscript, and provided critical intellectual input. All authors read and approved the final manuscript.

References

1. Saini V, Guada L, Yavagal D R. Global Epidemiology of Stroke and Access to Acute Ischemic Stroke Interventions[J]. *Neurology*, 2021,97(20 Suppl 2):S6-S16.
2. El A M, Wegener S. Improving Cerebral Blood Flow after Arterial Recanalization: A Novel Therapeutic Strategy in Stroke[J]. *Int J Mol Sci*, 2017,18(12).
3. Rabinstein A A. Update on Treatment of Acute Ischemic Stroke[J]. *Continuum (Minneap Minn)*, 2020,26(2):268-286.
4. Liu J, Zhou Y, Zhang L, et al. Balloon guide catheters for endovascular thrombectomy in patients with acute ischaemic stroke due to large-vessel occlusion in China (PROTECT-MT): a multicentre, open-label, blinded-endpoint, randomised controlled trial[J]. *Lancet*, 2024,404(10468):2165-2174.
5. French K F, White J, Hoesch R E. Treatment of intracerebral hemorrhage with tranexamic acid after thrombolysis with tissue plasminogen activator[J]. *Neurocrit Care*, 2012,17(1):107-111.

6. Albers G W, Olivot J M. Intravenous alteplase for ischaemic stroke[J]. *Lancet*, 2007,369(9558):249-250.
7. Li G, Wang C, Wang S, et al. Tenecteplase in Ischemic Stroke: Challenge and Opportunity[J]. *Neuropsychiatr Dis Treat*, 2022,18:1013-1026.
8. Yogendrakumar V, Churilov L, Mitchell P J, et al. Safety and Efficacy of Tenecteplase and Alteplase in Patients With Tandem Lesion Stroke: A Post Hoc Analysis of the EXTEND-IA TNK Trials[J]. *Neurology*, 2023,100(18):e1900-e1911.
9. Alkarithi G, Duval C, Shi Y, et al. Thrombus Structural Composition in Cardiovascular Disease[J]. *Arterioscler Thromb Vasc Biol*, 2021,41(9):2370-2383.
10. Dumitriu L D, Reymond P, Brina O, et al. Spatial heterogeneity of occlusive thrombus in acute ischemic stroke: A systematic review[J]. *J Neuroradiol*, 2023,50(3):352-360.
11. Jolugbo P, Ariens R. Thrombus Composition and Efficacy of Thrombolysis and Thrombectomy in Acute Ischemic Stroke[J]. *Stroke*, 2021,52(3):1131-1142.
12. Di Meglio L, Desilles J P, Ollivier V, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis[J]. *Neurology*, 2019,93(18):e1686-e1698.
13. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria[J]. *Science*, 2004,303(5663):1532-1535.
14. Jorch S K, Kubes P. An emerging role for neutrophil extracellular traps in noninfectious disease[J]. *Nat Med*, 2017,23(3):279-287.
15. Longstaff C, Varju I, Sotonyi P, et al. Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, and histones[J]. *J Biol Chem*, 2013,288(10):6946-6956.
16. de Bont C M, Boelens W C, Pruijn G. NETosis, complement, and coagulation: a triangular relationship[J]. *Cell Mol Immunol*, 2019,16(1):19-27.
17. Noubouossie D F, Whelihan M F, Yu Y B, et al. In vitro activation of coagulation by human neutrophil DNA and histone proteins but not neutrophil extracellular traps[J]. *Blood*, 2017,129(8):1021-1029.
18. Pena-Martinez C, Duran-Laforet V, Garcia-Culebras A, et al. Pharmacological Modulation of Neutrophil Extracellular Traps Reverses Thrombotic Stroke tPA (Tissue-Type Plasminogen Activator) Resistance[J]. *Stroke*, 2019,50(11):3228-3237.
19. Li C, Xing Y, Zhang Y, et al. Neutrophil Extracellular Traps Exacerbate Ischemic Brain Damage[J]. *Mol Neurobiol*, 2022,59(1):643-656.
20. Laridan E, Denorme F, Desender L, et al. Neutrophil extracellular traps in ischemic stroke thrombi[J]. *Ann Neurol*, 2017,82(2):223-232.
21. Ducroux C, Di Meglio L, Loyau S, et al. Thrombus Neutrophil Extracellular Traps Content Impair tPA-Induced Thrombolysis in Acute Ischemic Stroke[J]. *Stroke*, 2018,49(3):754-757.
22. Xu S, Yang Z, Jia J, et al. Enhancement of Tenecteplase Thrombolytic Actions Ex Vivo and In Vivo by Deoxyribonuclease I[J]. *J Am Heart Assoc*, 2025,14(15):e39199.

23. Li X, Wu F, Yu D, et al. Archaea-inspired deoxyribonuclease I liposomes prevent multiple organ dysfunction in sepsis[J]. *J Control Release*, 2025,380:1109-1126.
24. Filipczak N, Li X, Saawant G R, et al. Antibody-modified DNase I micelles specifically recognize the neutrophil extracellular traps (NETs) and promote their degradation[J]. *J Control Release*, 2023,354:109-119.
25. Phan A, Doonan C J, Uribe-Romo F J, et al. Synthesis, structure, and carbon dioxide capture properties of zeolitic imidazolate frameworks[J]. *Acc Chem Res*, 2010,43(1):58-67.
26. Zhang S, Peng B, Chen Z, et al. Brain-targeting, acid-responsive antioxidant nanoparticles for stroke treatment and drug delivery[J]. *Bioact Mater*, 2022,16:57-65.
27. Wang Y, Zeng M, Fan T, et al. Biomimetic ZIF-8 Nanoparticles: A Novel Approach for Biomimetic Drug Delivery Systems[J]. *Int J Nanomedicine*, 2024,19:5523-5544.
28. Zhang S, Peng B, Chen Z, et al. Brain-targeting, acid-responsive antioxidant nanoparticles for stroke treatment and drug delivery[J]. *Bioact Mater*, 2022,16:57-65.
29. Li X, Peng X, Zoulikha M, et al. Multifunctional nanoparticle-mediated combining therapy for human diseases[J]. *Signal Transduct Target Ther*, 2024,9(1):1.
30. Tang L, Fu C, Zhang A, et al. Harnessing nanobiotechnology for cerebral ischemic stroke management[J]. *Biomater Sci*, 2023,11(3):791-812.
31. Vijayan V, Uthaman S, Park I. Cell Membrane Coated Nanoparticles: An Emerging Biomimetic Nanoplatfrom for Targeted Bioimaging and Therapy[J]. *Advances in experimental medicine and biology*, 2018,1064:45-59.
32. Chai Y, Shangguan L, Yu H, et al. Near Infrared Light-Activatable Platelet-Mimicking NIR-II NO Nano-Prodrug for Precise Atherosclerosis Theranostics[J]. *Adv Sci (Weinh)*, 2024,11(3):e2304994.
33. Cai W, Zhang W, Chen Z. Magnetic Fe(3)O(4)@ZIF-8 nanoparticles as a drug release vehicle: pH-sensitive release of norfloxacin and its antibacterial activity[J]. *Colloids and surfaces. B, Biointerfaces*, 2023,223:113170.
34. Zor F, Selek F N, Orlando G, et al. Biocompatibility in regenerative nanomedicine[J]. *Nanomedicine (London, England)*, 2019,14(20):2763-2775.
35. Dimitrov J D, Roumenina L T, Perrella G, et al. Basic Mechanisms of Hemolysis-Associated Thrombo-Inflammation and Immune Dysregulation[J]. *Arterioscler Thromb Vasc Biol*, 2023,43(8):1349-1361.
36. Vogel S, Bodenstein R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis[J]. *The Journal of clinical investigation*, 2015,125(12):4638-4654.
37. Koto T, Takubo K, Ishida S, et al. Hypoxia disrupts the barrier function of neural blood vessels through changes in the expression of claudin-5 in endothelial cells[J]. *The American journal of pathology*, 2007,170(4):1389-1397.
38. Zhou Y, Xu Z, Liu Z. Impact of Neutrophil Extracellular Traps on Thrombosis Formation: New Findings and Future Perspective[J]. *Front Cell Infect Microbiol*, 2022,12:910908.

39. Marta-Enguita J, Machado F, Orbe J, et al. Thrombus composition and its implication in ischemic stroke assessment and revascularization treatments[J]. *Neurologia (Engl Ed)*, 2025,40(1):77-88.
40. Van de Werf F, Barron H V, Armstrong P W, et al. Incidence and predictors of bleeding events after fibrinolytic therapy with fibrin-specific agents: a comparison of TNK-tPA and rt-PA[J]. *European heart journal*, 2001,22(24):2253-2261.
41. Hu C M, Fang R H, Wang K C, et al. Nanoparticle biointerfacing by platelet membrane cloaking[J]. *Nature*, 2015,526(7571):118-121.
42. Guo X, Hong T, Zang J, et al. Thrombus-specific/responsive biomimetic nanomedicine for spatiotemporal thrombolysis and alleviation of myocardial ischemia/reperfusion injury[J]. *J Nanobiotechnology*, 2022,20(1):531.
43. Quan X, Liang X, Ding Y, et al. Cryo-Shocked Platelet Coupled with ROS-Responsive Nanomedicine for Targeted Treatment of Thromboembolic Disease[J]. *ACS Nano*, 2023,17(7):6519-6533.
44. Wang L, Ashrafizadeh M, Sethi G, et al. pH-responsive biomimetic zeolitic imidazolate framework-based nanoparticles for co-delivery of cetuximab and siRNA in synergistic therapy of laryngeal squamous cell carcinoma[J]. *Journal of pharmaceutical analysis*, 2025,15(7):101203.
45. Li H, Xie X, Zhang Y, et al. MOF-Engineered Platelet-Mimicking Nanocarrier-Encapsulated Cascade Enzymes for ROS Scavenging and Anti-Inflammation in Cerebral Ischemia-Reperfusion Injury[J]. *Pharmaceutics*, 2025,17(11):1478.
46. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery[J]. *Nat Biotechnol*, 2015,33(9):941-951.
47. Wei Z, Chen Z, Zhao Y, et al. Mononuclear phagocyte system blockade using extracellular vesicles modified with CD47 on membrane surface for myocardial infarction reperfusion injury treatment[J]. *Biomaterials*, 2021,275:121000.
48. Saver J L, Goyal M, van der Lugt A, et al. Time to Treatment With Endovascular Thrombectomy and Outcomes From Ischemic Stroke: A Meta-analysis[J]. *JAMA*, 2016,316(12):1279-1288.
49. Bacha Z, Javed J, Sheraz M, et al. Efficacy and Safety of Tenecteplase in Acute Ischemic Stroke: A Meta-Analysis of Randomized Controlled Trials[J]. *Brain and behavior*, 2025,15(9):e70791.
50. Zaidat O O, Yoo A J, Khatri P, et al. Recommendations on angiographic revascularization grading standards for acute ischemic stroke: a consensus statement[J]. *Stroke*, 2013,44(9):2650-2663.
51. Catanese L, Tarsia J, Fisher M. Acute Ischemic Stroke Therapy Overview[J]. *Circ Res*, 2017,120(3):541-558.
52. Klopff J, Brostjan C, Eilenberg W, et al. Neutrophil Extracellular Traps and Their Implications in Cardiovascular and Inflammatory Disease[J]. *International journal of molecular sciences*, 2021,22(2):559.
53. Zhang Y, Zhang Q, Li C, et al. Advances in cell membrane-based biomimetic nanodelivery systems for natural products[J]. *Drug delivery*, 2024,31(1):2361169.

Figures

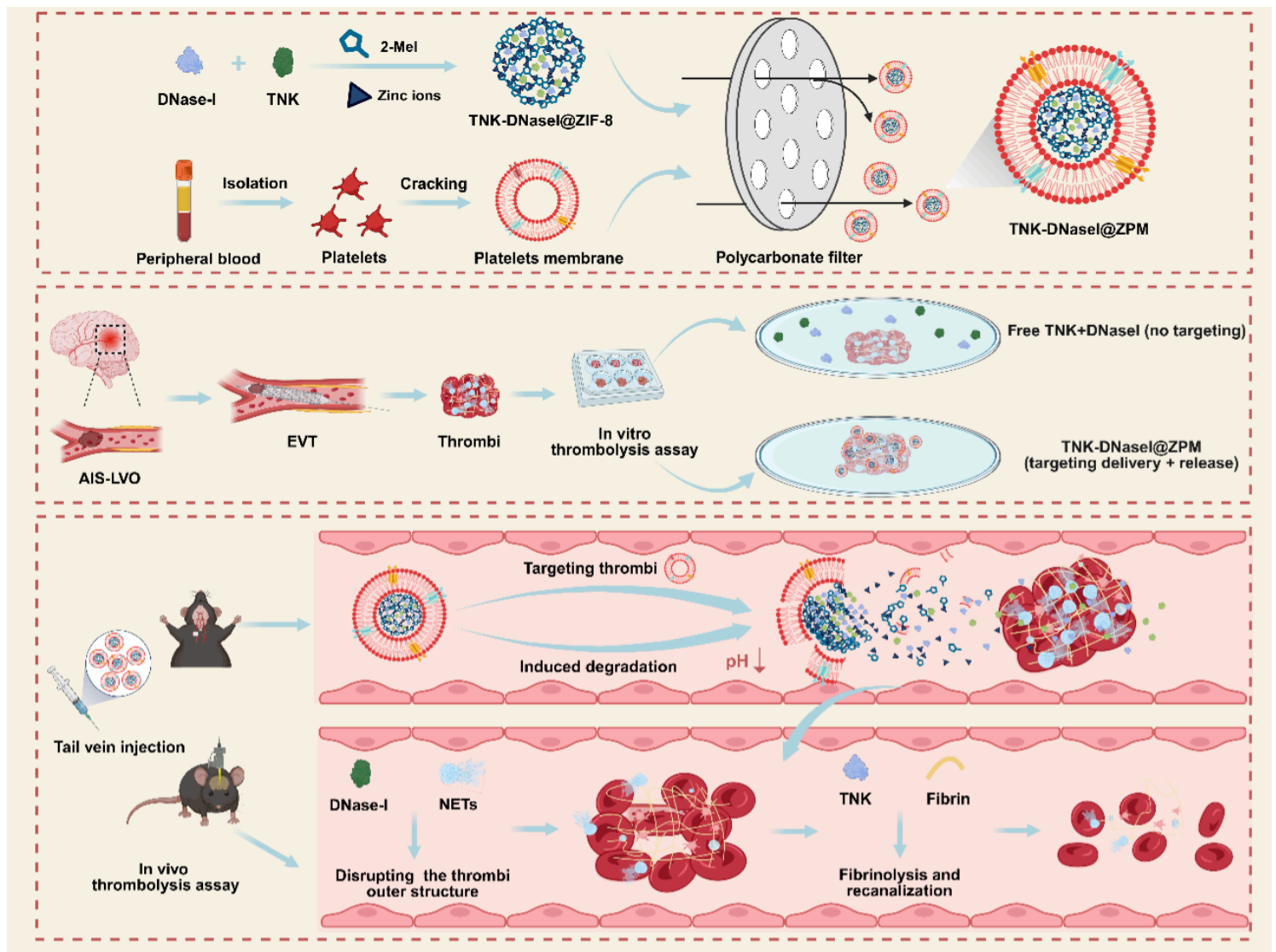


Figure 1

Schematic illustration of the TNK-DNaseI@ZPM nanosystem for targeted thrombolytic therapy. The biomimetic nanoparticle (TD@ZPM) is engineered with ZIF-8 as the core to co-encapsulate TNK and DNase-I, followed by platelet membrane (PM) coating to confer thrombus-targeting capability. Upon exposure to the acidic microenvironment (pH 6.4) at the thrombus site, the ZIF-8 framework undergoes pH-responsive degradation, enabling on-demand release of TNK and DNase-I. The released drugs exert a synergistic effect: TNK dissolves fibrin, and DNase-I degrades neutrophil extracellular traps (NETs), which jointly promote effective thrombolysis in both in vitro experiments (AIS-LVO thrombi and artificial blood clots as samples) and in vivo experiments (the FeCl₃-induced carotid thrombosis and the photothrombotic stroke model), ultimately achieving successful vascular recanalization.

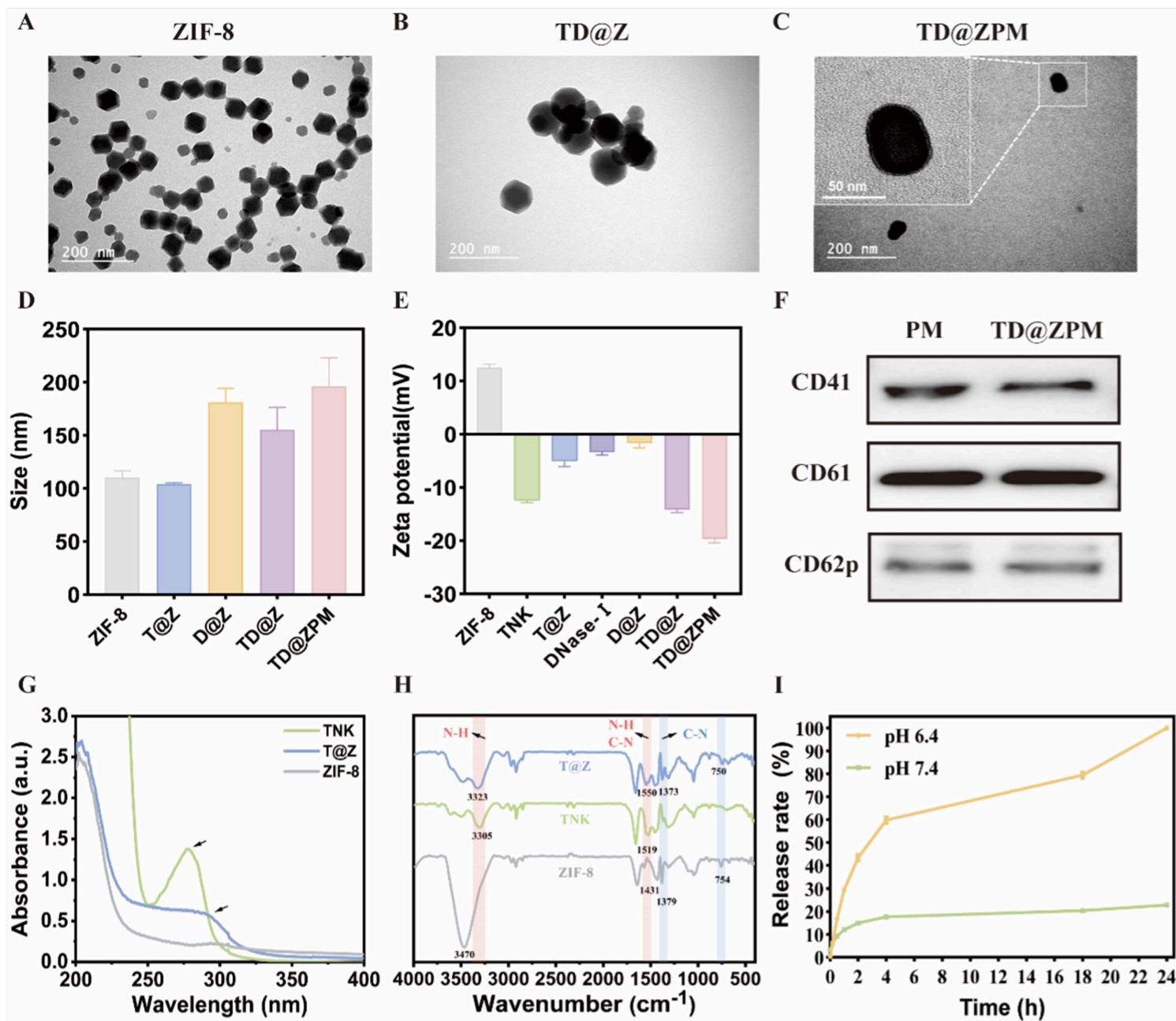


Figure 2

Characterization of nanoparticles. (A-C) Representative TEM images of ZIF-8, TD@Z and TD@ZPM, scale bar = 200 nm. (D) Hydrodynamic diameters of ZIF-8, T@Z, DNase-I@ZIF-8 (D@Z), TD@Z and TD@ZPM. (E) Zeta potentials of ZIF-8, TNK, T@Z, DNase-I, D@Z, TD@Z and TD@ZPM. (F) Western blot analysis confirming the retention of PM proteins (CD41, CD61, CD62p) in both purified PM and TD@ZPM. (G) UV-vis absorption spectra of ZIF-8, TNK and T@Z. (H) FTIR of ZIF-8, TNK and T@Z. (I) In vitro cumulative release profile of TNK from T@Z under pH 7.4 and 6.4; (n = 3).

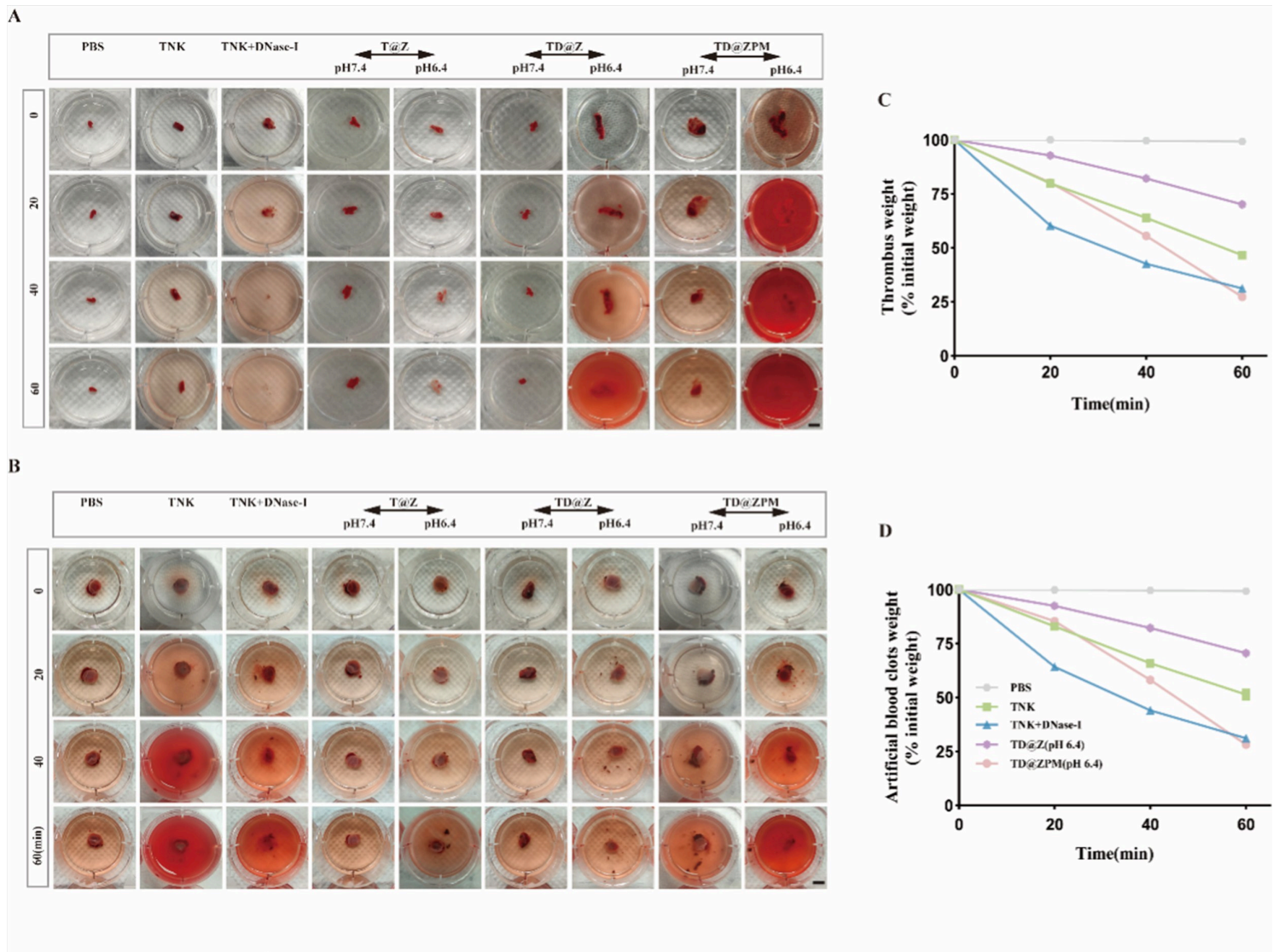


Figure 3

In vitro thrombolysis evaluation. (A-B) Representative images of AIS-LVO thrombi (A) and artificial clots (B) following various treatments. (C) Quantitative analysis of residual thrombus weight from AIS-LVO patients. At 60 minutes, TNK+DNase-I vs TNK, $P < 0.001$; TD@ZPM vs TNK+DNase-I, $P = 0.13$; TD@ZPM vs all other groups, $P < 0.0001$. (D) Quantitative analysis of residual weight for artificial clots. At 60 minutes, TNK+DNase-I vs TNK, $P < 0.01$; TD@ZPM vs TNK+DNase-I, $P < 0.05$; TD@ZPM vs all other groups, $P < 0.0001$; ($n = 6$).

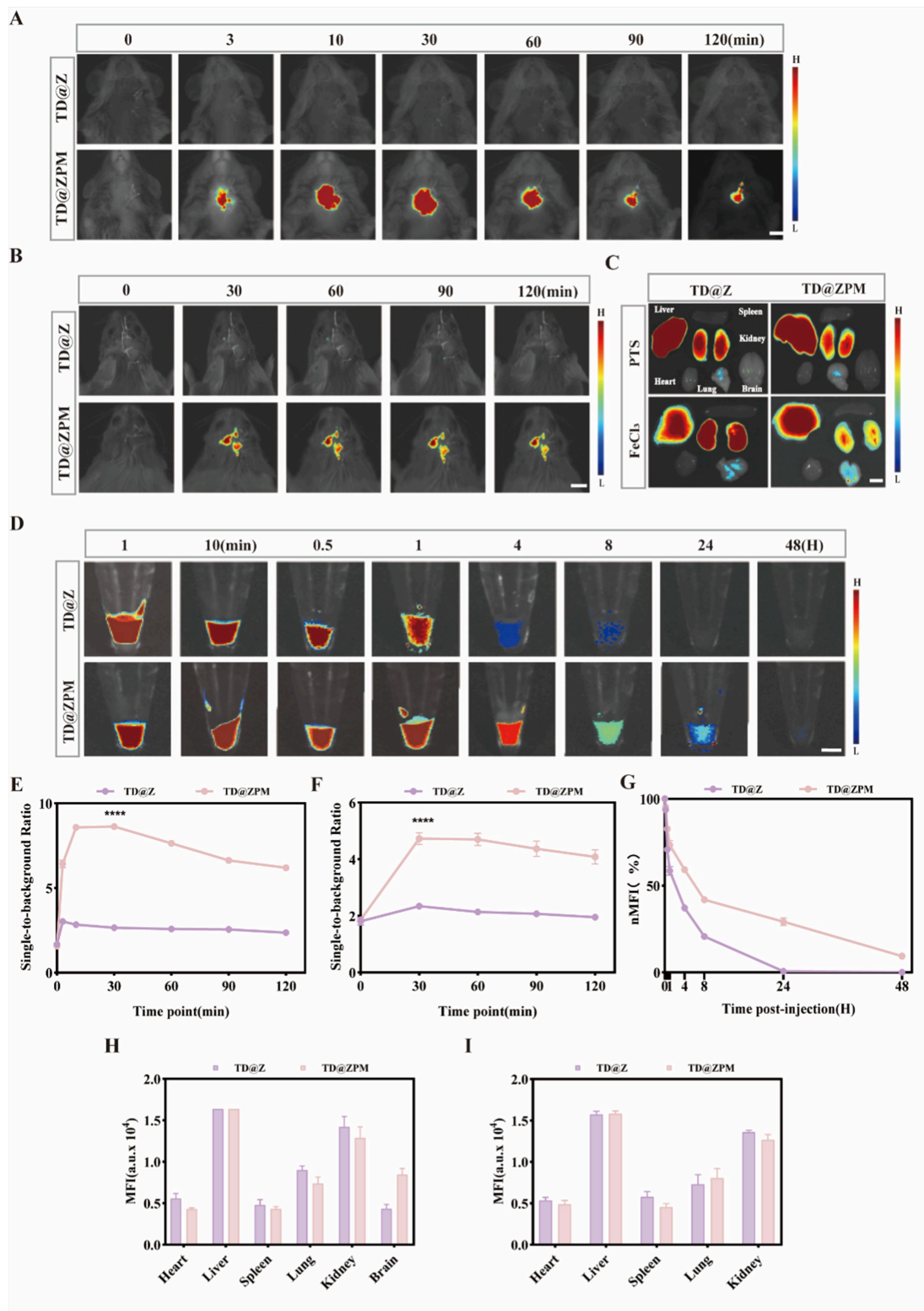


Figure 4

Thrombus targeting, biodistribution and pharmacokinetics of TD@Z and TD@ZPM. (A-B) Whole-body NIR-II imaging showing time-dependent distribution in FeCl₃-induced carotid thrombosis (A) and PTS models (B). (C) In vitro NIR-II images of major organs harvested 2 h post-injection. (D) Serial fluorescent images of blood samples from healthy mice at indicated time points. (E-F) Quantitative signal-to-background ratios (SBR) at thrombus sites in FeCl₃-induced carotid thrombosis (E) and PTS (F) models.

(G) Pharmacokinetic profiles of TD@Z and TD@ZPM, presented as normalized blood fluorescence intensity versus time in healthy mice. (H-I) Mean fluorescence intensities of organs in PTS (H) and FeCl₃-induced carotid thrombosis (I) models; *****P* < 0.0001; (n = 3).

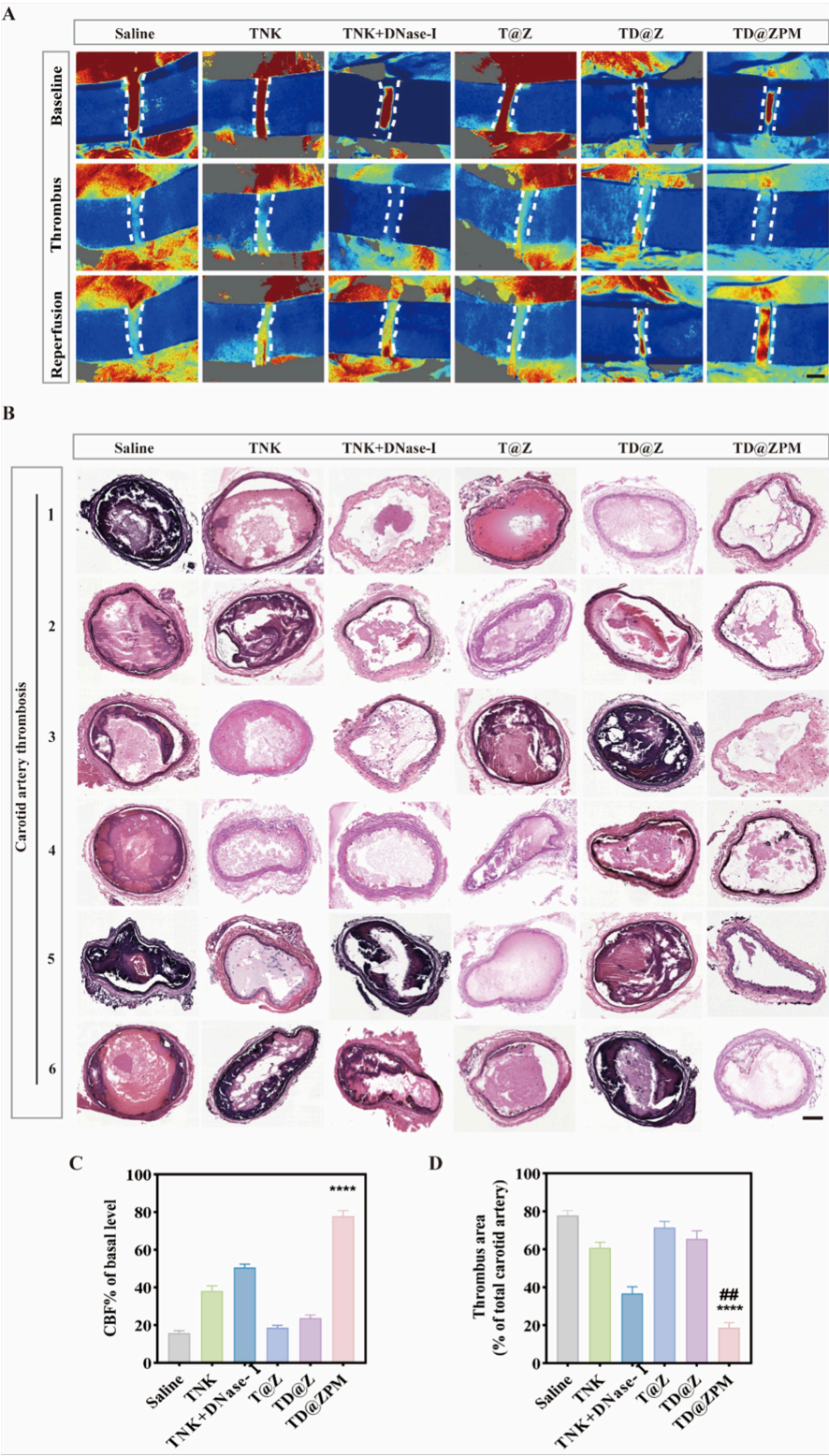


Figure 5

In vivo thrombolysis in the FeCl₃-induced carotid thrombosis model.(A) Representative LSCI showing blood flow recovery in occluded vessels after treatment. (B) HE-stained cross-sections of occluded vessels. (C) Quantitative analysis of relative carotid blood flow recovery over time. (D) Quantitative assessment of relative thrombus area; ##*P* < 0.01, *****P* < 0.0001; (n = 6).

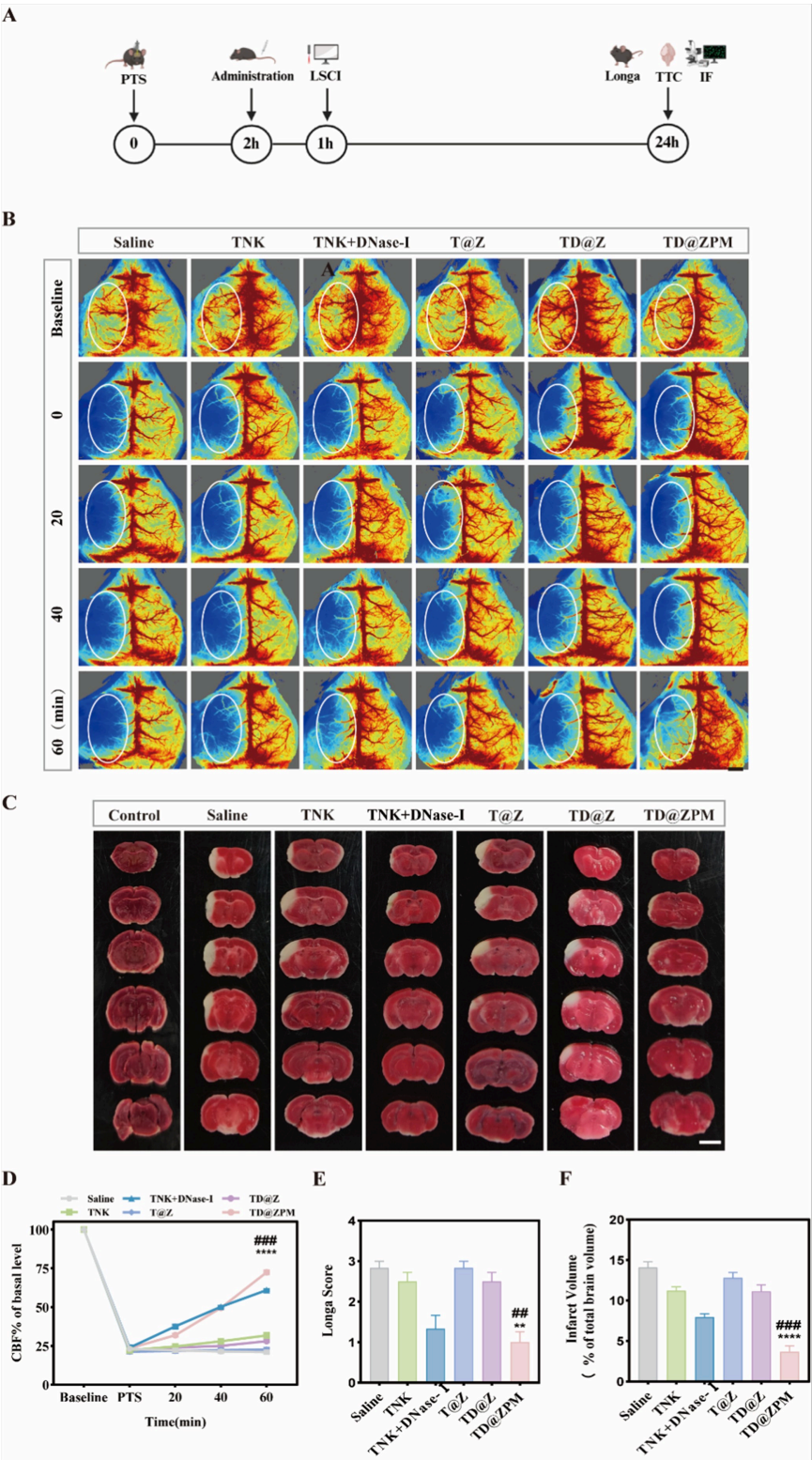


Figure 6

In vivo thrombolysis in a PTS model. (A) Illustration of the procedures in PTS model. (B) Representative LSCI of CBF at selected time points within 60 minutes. (C) Representative triphenyltetrazole chloride (TTC)-stained brain sections at 24 h post-treatment. (D) Quantitative analysis of relative CBF over time. (E) Quantification of cerebral infarct volume. (F) Neurological deficit scores assessed by the Longa scale at 24 h; ##*P* < 0.01, ***P* < 0.01, ###*P* < 0.001, *****P* < 0.0001; (n = 6).

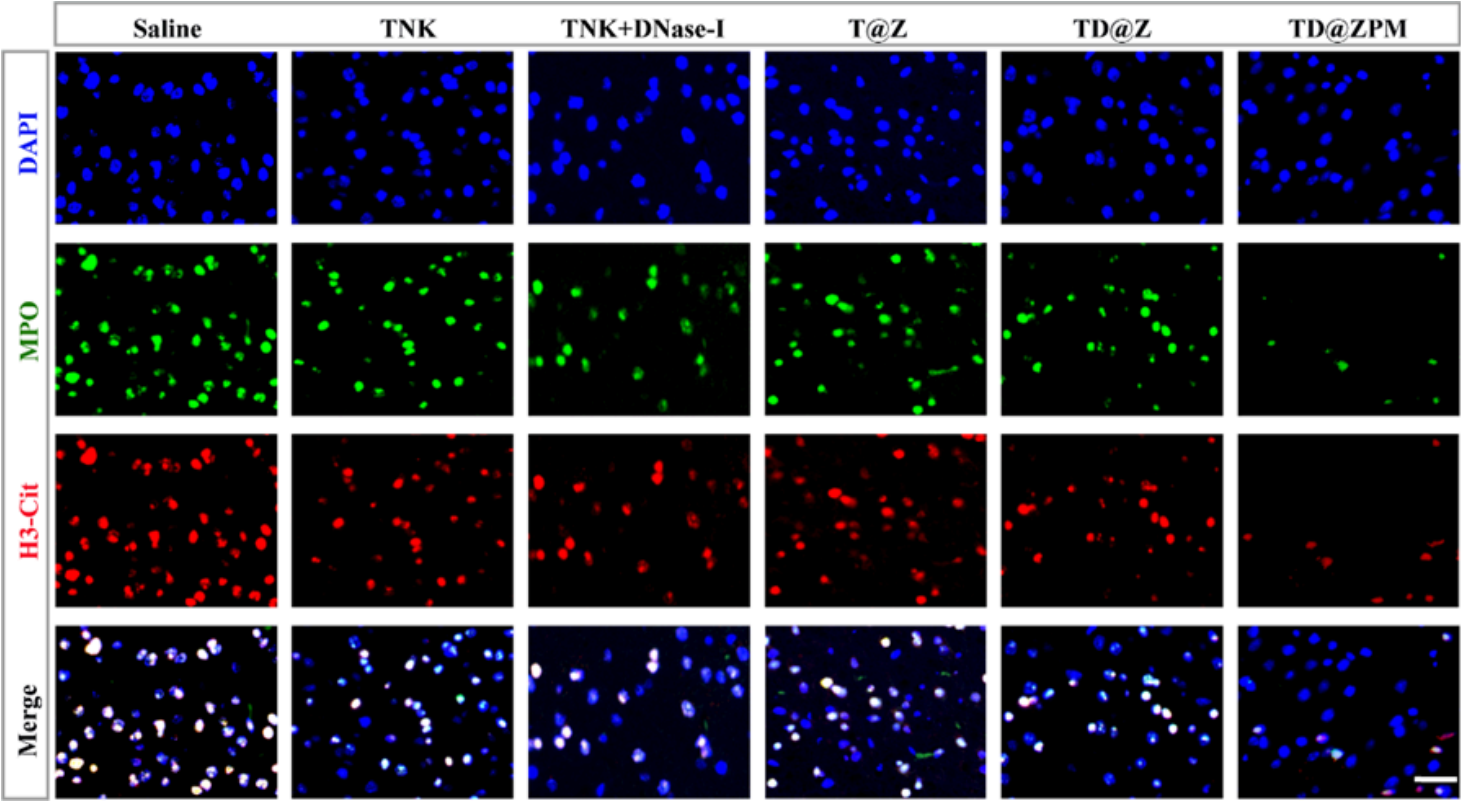


Figure 7

NETs in the ischemic area of PTS mice at 24 h post-treatment. Co-localization of neutrophil marker MPO (green), NET marker H3Cit (red), and DAPI (blue) indicates NETs; scale bar = 20 μm; (n = 6).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupportingInformation.docx](#)