

ILLUSTRATED REVIEW

Fibrinolysis: an illustrated review

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Funding information

NIH R00HL148646-01 (V.T.), New Jersey Commission for Cancer Research COCR22PRF010 (R.R.), NIH T32 GM135141 (R.R.), NIH R15HL148842 (N.E.H.), and R15HL150666 (N.E.H.)

Handling Editor: M Sholzberg

Abstract

In response to vessel injury (or other pathological conditions), the hemostatic process is activated, resulting in a fibrous, cellular-rich structure commonly referred to as a blood clot. Succeeding the clot's function in wound healing, it must be resolved. This illustrated review focuses on fibrinolysis—the degradation of blood clots or thrombi. Fibrin is the main mechanical and structural component of a blood clot, which encases the cellular components of the clot, including platelets and red blood cells. Fibrinolysis is the proteolytic degradation of the fibrin network that results in the release of the cellular components into the bloodstream. In the case of thrombosis, fibrinolysis is required for restoration of blood flow, which is accomplished clinically through exogenously delivered lytic factors in a process called external lysis. Fibrinolysis is regulated by plasminogen activators (tissue-type and urokinase-type) that convert plasminogen into plasmin to initiate fiber lysis and lytic inhibitors that impede this lysis (plasminogen activator inhibitors, alpha 2-antiplasmin, and thrombin activatable fibrinolysis inhibitor). Furthermore, the network structure has been shown to regulate lysis: thinner fibers and coarser clots lyse faster than thicker fibers and finer clots. Clot contraction, a result of platelets pulling on fibers, results in densely packed red blood cells (polyhedrocytes), reduced permeability to fibrinolytic factors, and increased fiber tension. Extensive research in the field has allowed for critical advancements leading to improved thrombolytic agents. In this review, we summarize the state of the field, highlight gaps in knowledge, and propose future research questions.

KEY WORDS

blood, blood coagulation, blood clot, clot lysis time, fibrinolysis, fibrin

Essentials

- Fibrinolysis is the degradation of the fibrin network of a blood clot.
- Fibrinolysis is required to achieve hemostatic balance.
- Fibrinolysis can occur naturally in the body or with delivery of pharmacological agents.
- Rate of fibrinolysis is affected by the fibrin structure and the clot's cellular components.

Rebecca A. Risman and Nicholas C. Kirby contributed equally to this study.

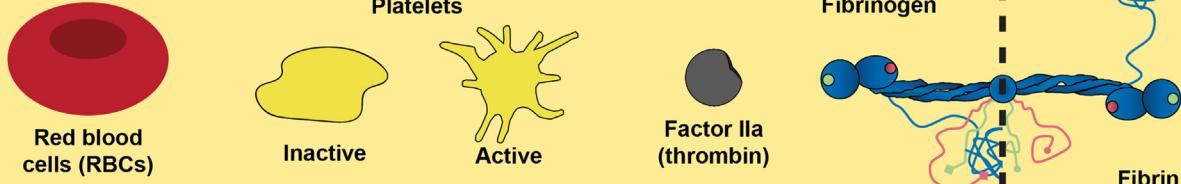
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Blood coagulation key players

"Blood is composed of a variety of cells and proteins involved in clot formation and dissolution. Below are components associated with fibrin fiber formation, as well as fibrinolysis activation and inhibition."

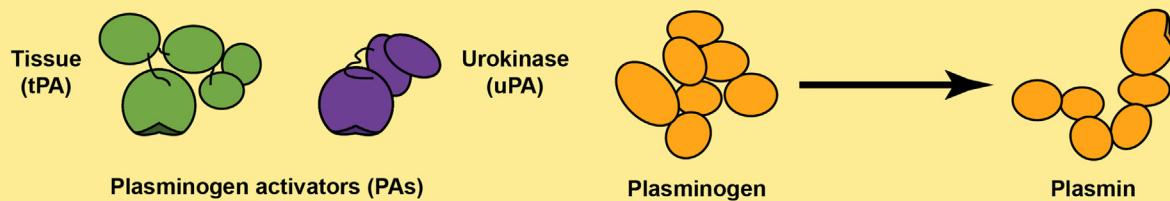


Clot components



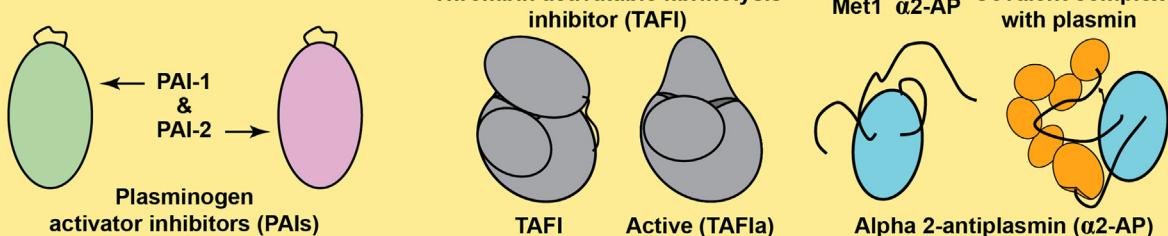
Clots are composed of fibrin and its precursor fibrinogen (which provide the structural and mechanical stability of a clot), red blood cells (which take up the majority of the volume), and platelets (which form the primary hemostatic plug, release numerous coagulation factors, and attach to and pull on fibrin fibers). Coagulation occurs when thrombin converts fibrinogen into fibrin.

Lysis activators/enzymes



Fibrin networks are degraded by plasmin, the activated form of plasminogen. Plasminogen conversion occurs by plasminogen activators (PAs), namely tissue-type PA (tPA) and urokinase-type PA (uPA), converting inactive Glu-plasminogen into active Lys-plasmin. Plasmin then cleaves fibrin at specific lysine residues, creating a positive feedback loop by exposing C-terminal lysines to which more lytic enzymes can bind.

Lysis inhibitors



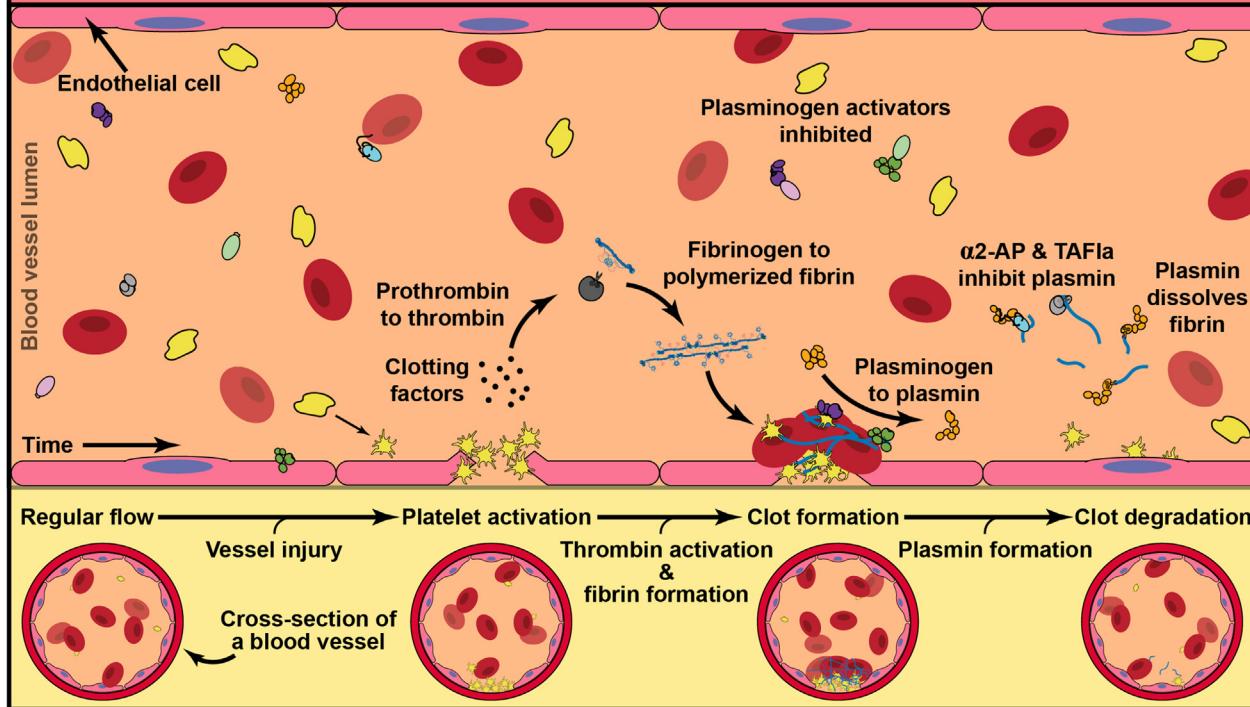
There are many types of fibrinolytic inhibitors. This review will focus on three common lysis inhibitors: plasminogen activator inhibitors (PAIs 1 & 2), alpha 2-antiplasmin (α 2-AP), and thrombin activatable fibrinolysis inhibitor (TAFI). Direct inhibitors (PAIs and α 2-AP) target specific fibrinolytic enzymes while indirect inhibitors (TAFI) impede binding of fibrinolytic enzymes to fibrin.

Throughout this review, we will include color-coded magnifying glasses (i.e., to direct you to see the factor in action in the microscale overview. We also include thought bubbles to propose interesting areas for future work.

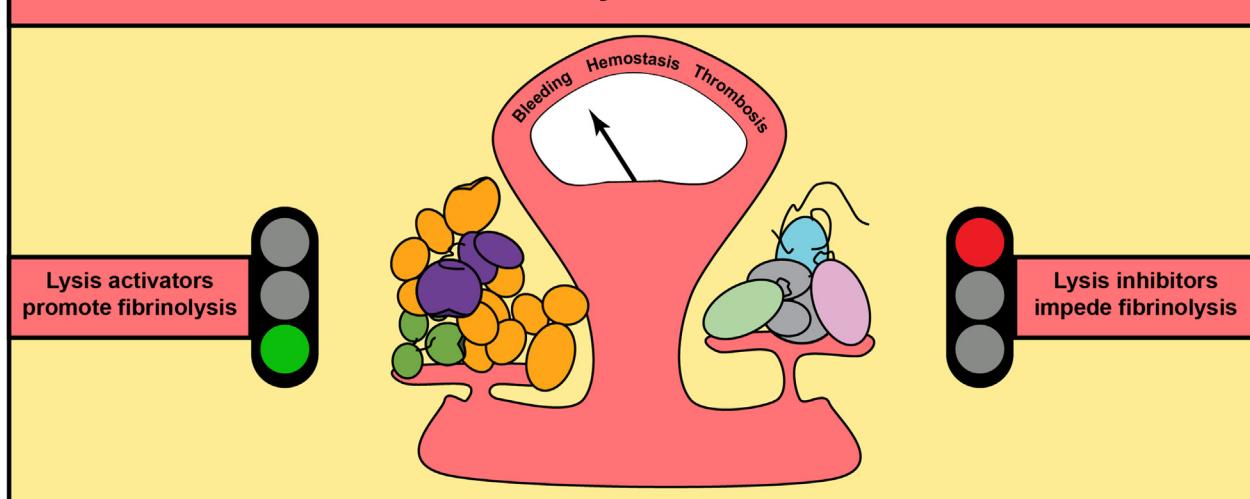
Fibrinolysis¹⁻³

The enzymatic degradation of blood clots

Macroscale overview of hemostasis and fibrinolysis



Fibrinolytic balance

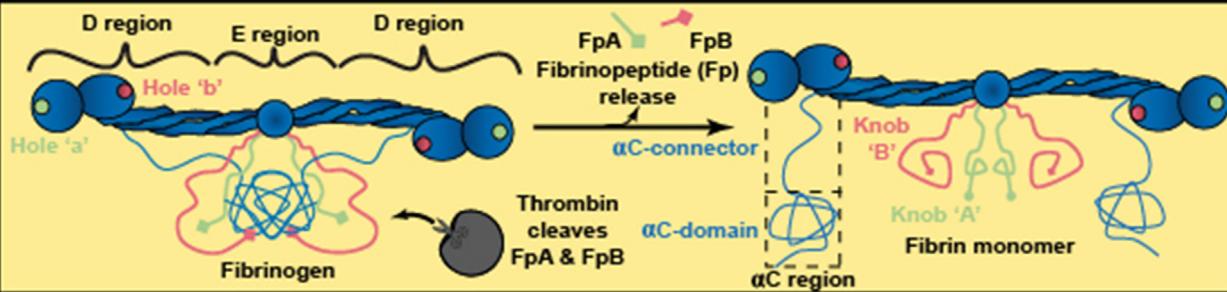


"Hemostasis requires a balance between bleeding and thrombosis, which is regulated by lysis activators and inhibitors. Bleeding occurs when the levels of lysis activators "outweigh" those of lysis inhibitors, resulting in increased fibrinolysis. Thrombosis occurs when the levels of lysis inhibitors "outweigh" those of lysis activators, resulting in decreased fibrinolysis and more persistent clot formation. This review will discuss specific factors involved in keeping the fibrinolytic scale balanced to avoid fatal conditions."

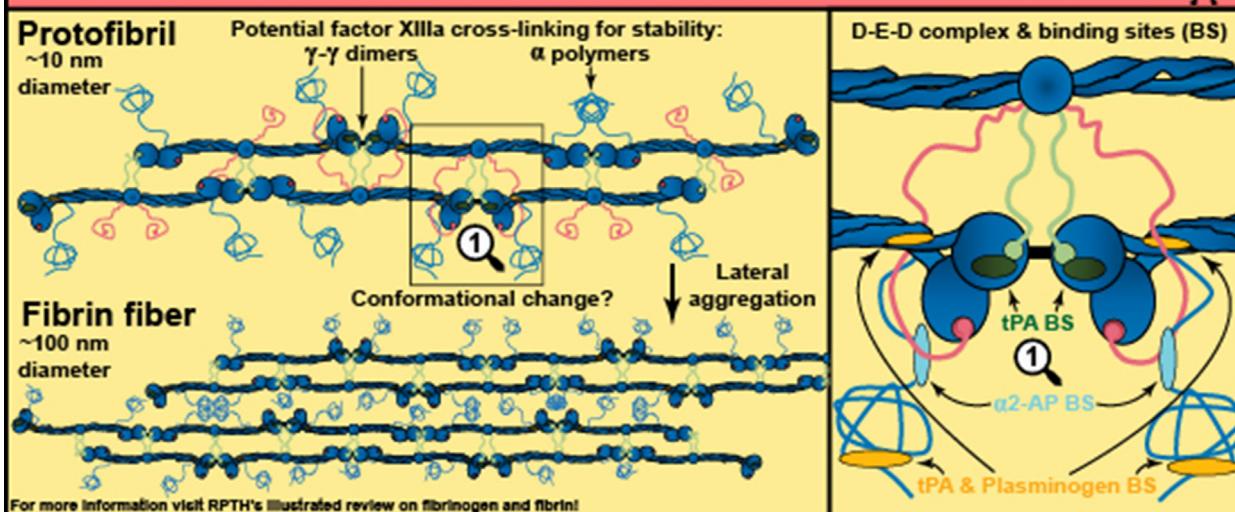


Fiber polymerization and digestion⁴⁻¹¹

Formation fundamentals⁴⁻¹⁰

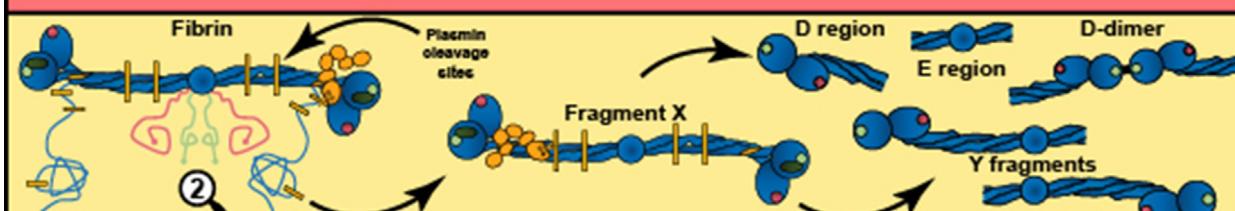


"Thrombin cleavage of fibrinopeptides A and B (FpA/B) converts fibrinogen into fibrin, exposing knobs 'A' and 'B' for binding to holes 'a' and 'b', respectively (1). Fibrin monomers interact in a half-staggered manner to form protofibrils that laterally aggregate into a fibrin network stabilized by FXIIIa cross-linking. Network digestion into smaller fibrin-based molecules (see FDP's below) occurs at specific molecular locations (2)."



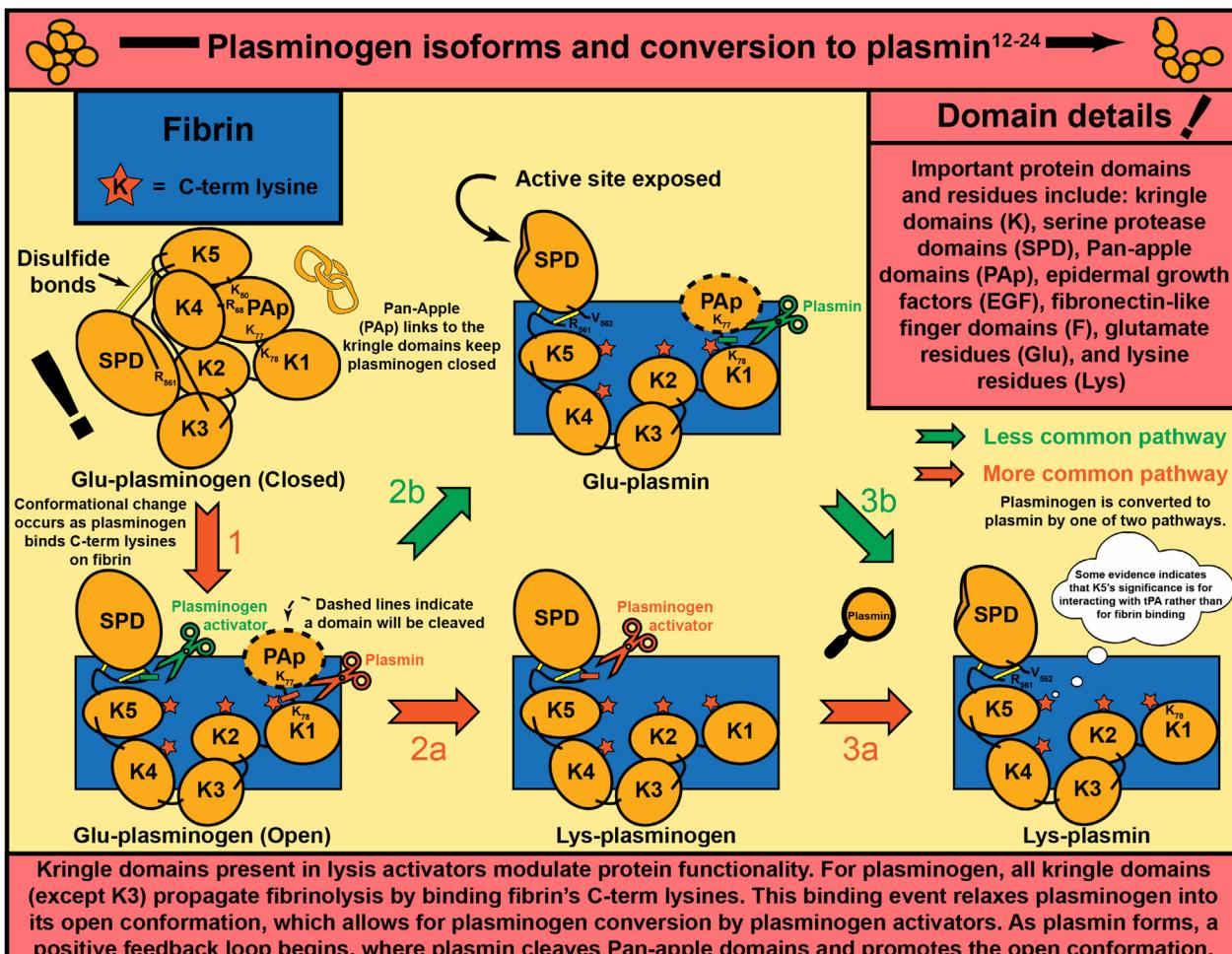
"It is hypothesized that a conformational change of the β nodules reveals cryptic plasminogen/tPA binding sites (α 148-160) after fibrinogen to fibrin conversion. Similarly, a change between β and γ nodules may uncover tPA-specific binding sites (γ 312-324). Although the above binding sites are involved in fibrinolysis activation, α 2-AP binding sites on the α C region exhibit inhibition of fibrinolysis by plasmin deactivation."

Fibrin degradation products (FDPs)¹¹

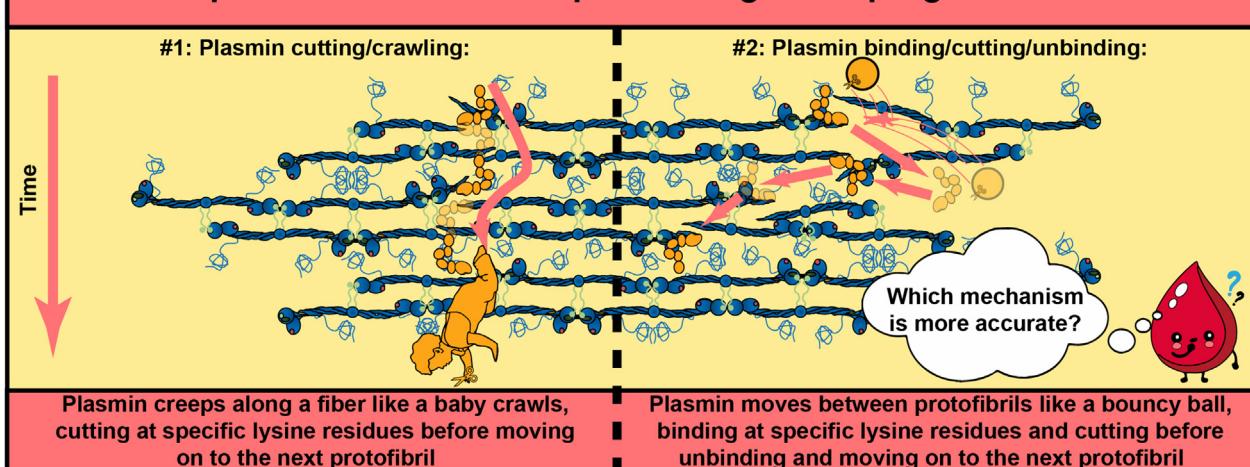


Plasmin-mediated fibrinolysis results in fibrin degradation products (FDPs). The α C region is fully removed by cleavage at residues α 230 and α 206 to give fragment X. This is further degraded at points along the coiled-coil region, where degradation of FXIIIa ligated fibrin may result in the release of D-dimer fragments.

Plasmin-mediated fibrinolysis¹²⁻²⁹



Proposed mechanisms of plasmin digestion progression²⁵⁻²⁹

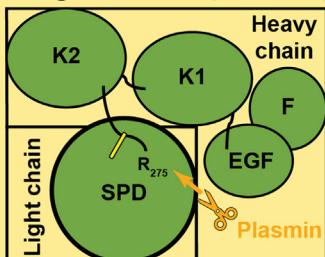


Plasminogen activators' role in fibrinolysis

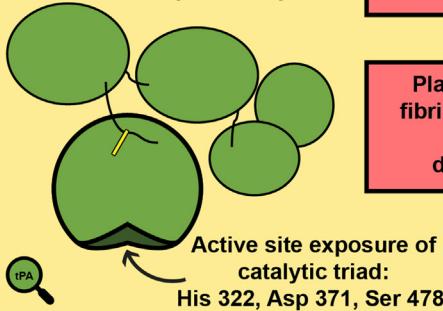
Plasminogen activators (PAs)³⁰⁻⁴⁰

Tissue-type PA (tPA)

Single chain (sc-tPA)



Two chain (tc-tPA)

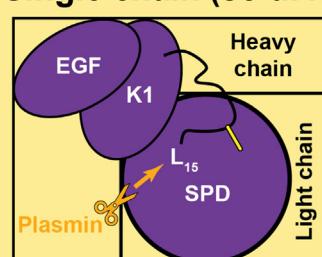


tPA is released from endothelial cells

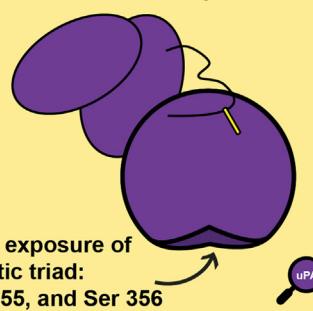
Elevated levels of uPA are associated with cancer

Urokinase-type PA (uPA)

Single chain (sc-uPA)



Two chain (tc-uPA)



tPA's finger domain (F) helps bind fibrin. Thus, tPA forms a trimeric complex with fibrin and plasminogen, whereas uPA only forms a dimeric complex with plasminogen.

The inactive single chain forms of tPA and uPA are converted to their active two chain forms by plasmin cleavage of distinct residues (tPA=Arg, uPA=Lys)

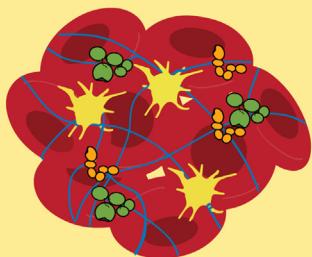
Plasminogen activators (PAs) initiate fibrinolysis by converting plasminogen into plasmin by cleaving a distinct arginine residue (R 561).

"tPA and uPA differ in their plasminogen conversion by targeting fibrin-bound or circulating plasminogen, respectively. tPA's fibrin specificity comes from the presence of a fibronectin-like finger domain (F), which uPA lacks. The two-chain forms of both PAs are more efficient than their single-chain forms, but the single-chain PAs are still functional. Though both PAs perform similar functions, tPA is more common."



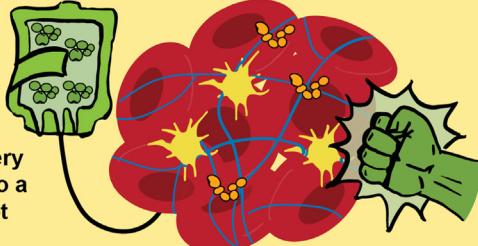
Fibrinolysis can occur in two ways, or in combination

Internal fibrinolysis



Physiological presence of lytic enzymes within a clot

External fibrinolysis



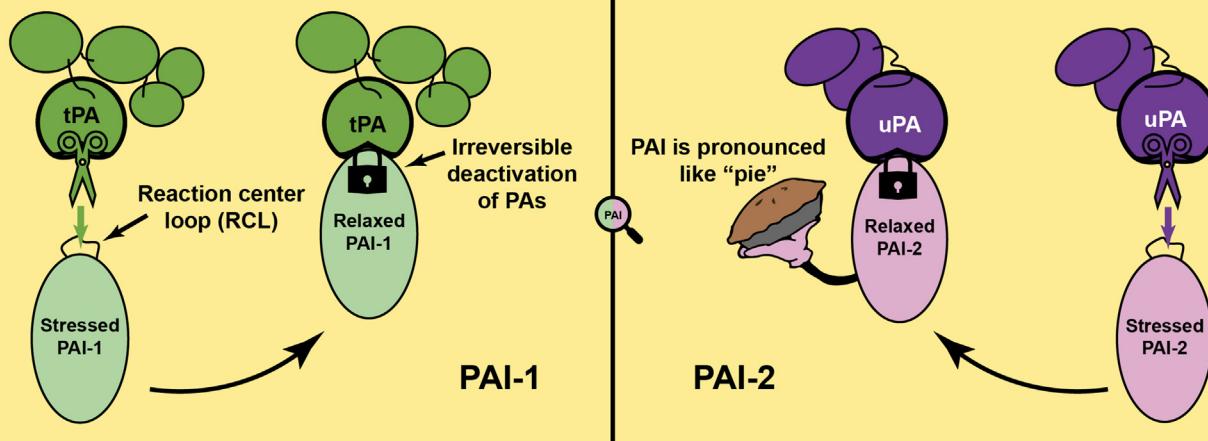
tPA is a "clot buster" for clots that cannot be degraded on their own

Fibrinolysis inhibitors⁴⁰⁻⁵⁹

Below is each inhibitor's proposed mechanism of action

Plasminogen activator inhibitors (PAIs)⁴¹⁻⁴⁷

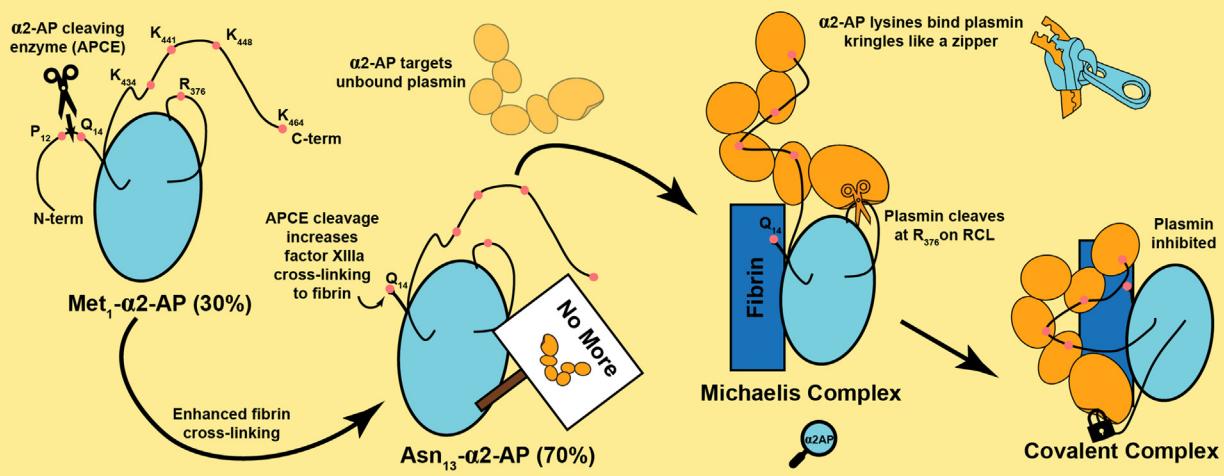
PAIs inhibit lysis by binding PAs and impairing their ability to activate plasminogen into plasmin to cleave fibrin



PAs cleave PAIs' RCLs and become covalently bound, thereby irreversibly inhibiting themselves in what is referred to as a "suicide mechanism." There are two PAI isoforms: PAI-1 and PAI-2. While PAI-1 is more potent and efficient than PAI-2, tPA and uPA are affected by each PAI differently. PAI-1 is more potent for tPA than uPA, while PAI-2 is more potent for uPA than tPA. PAI-1 levels are elevated from obesity, cancer, and even pregnancy.

α_2 -Antiplasmin⁴⁸⁻⁵²

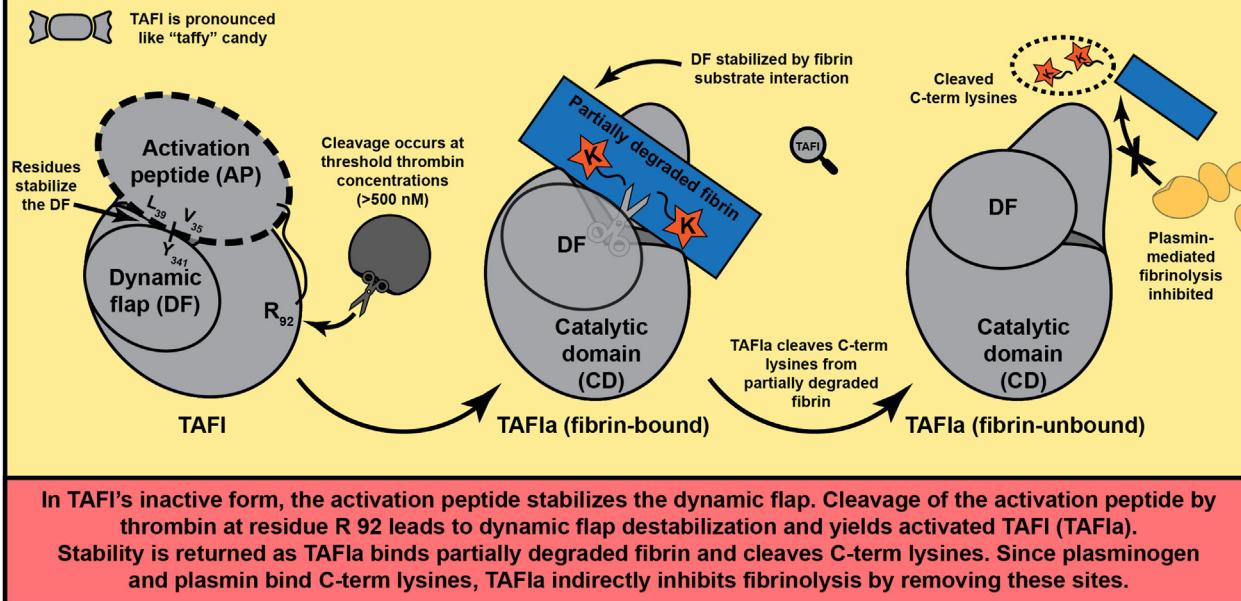
α_2 -AP limits lysis by binding to and inhibiting plasmin



α_2 -AP is covalently linked to fibrin by factor XIIIa, and is involved in the inhibition of plasmin-mediated fibrinolysis. Incorporation of α_2 -AP into the fibrin network is increased (13 times faster) after APCE cleaves N-term residues of Met1- α_2 -AP (30% of α_2 -AP in blood) to give Asn13- α_2 -AP (70% of α_2 -AP in blood). Covalently linked to α C residue Lys 303, α_2 -AP is situated to grab unbound plasmin molecules and inhibit further fibrinolysis.

Thrombin activatable fibrinolysis inhibitor (TAFI)⁵²⁻⁵⁸

TAFI inhibits lysis by cleaving plasmin binding sites on fibrin

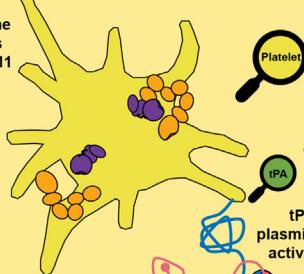


Microscale overview of fibrinolysis⁵⁹

Activation

Platelet surfaces promote the activation of lytic enzymes such as uPA. See pages 10-11 for more about platelets.

uPA-plasminogen activation



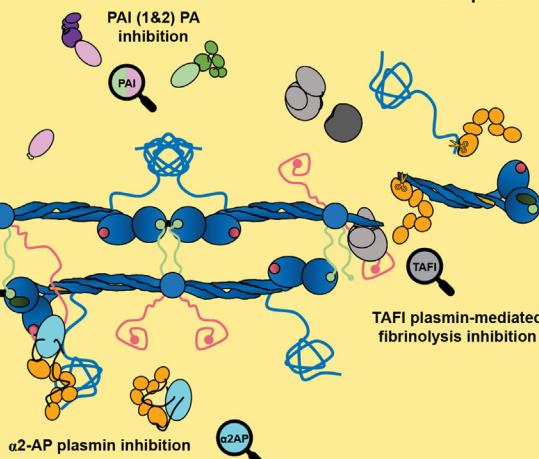
Plasmin-mediated degradation

Plasmin

Inhibition

TAFI: thrombin activatable fibrinolysis inhibitor
tPA: tissue plasminogen activator
uPA: urokinase plasminogen activator
PAI: plasminogen activator inhibitor
 α 2-AP: α 2-antiplasmin

PAI (1&2) PA inhibition



TAFI plasmin-mediated fibrinolysis inhibition

α 2-AP plasmin inhibition

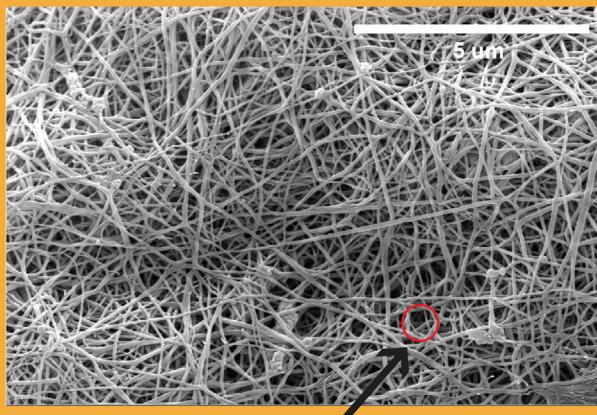
α 2AP

"As discussed on page 2, a balance of fibrinolytic activators and inhibitors are required to achieve hemostasis. The above graphic displays uPA, tPA, and plasmin in action to promote fibrinolysis (left) and PAIs, α 2-AP, and TAFI interacting with the activators to inhibit fibrinolysis (right)."



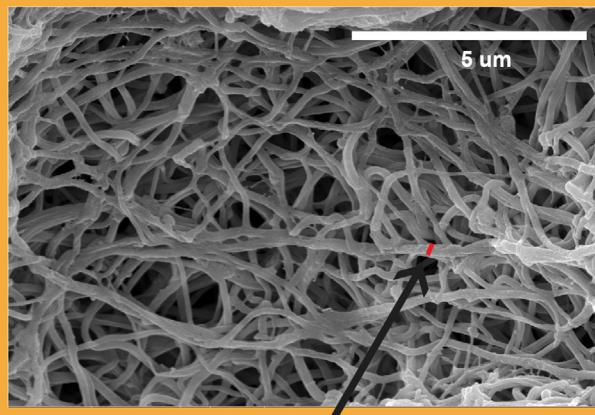
Influence of fibrin network structure on fibrinolysis⁶⁰⁻⁷²

Fine/densely packed fibrin network with thin individual fibers



The pore size of the fibrin network is associated with network density. The lytic enzymes diffuse or perfuse through this space.

Coarse/loosely packed fibrin network with thick individual fibers*



The diameter of the fibrin fiber is dependent on the lateral aggregation of protofibrils. Sometimes lateral aggregation occurs during the onset of lysis.

"Below, we represent fiber thickness by the width of the tree trunk and the packing density of the network by the number of trees that take up the same volume. The axe cutting down the trees is akin to plasmin lysing fibers."



Individual thin fibers lyse faster than individual thick fibers⁶⁰⁻⁶³



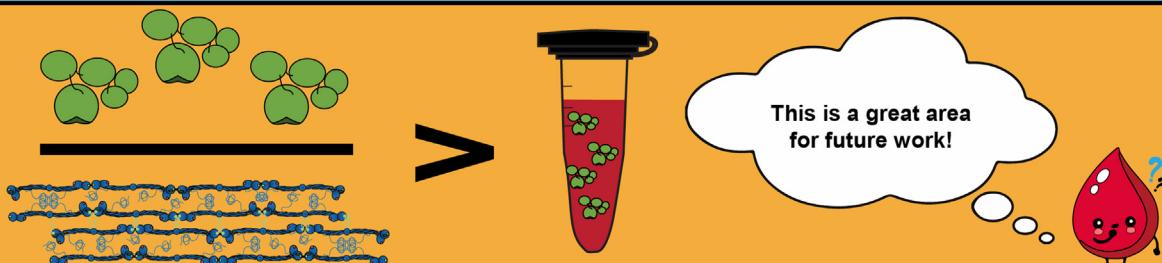
Thinner fibers have less width for plasmin/tPA to transversely cut.

Coarse clots lyse faster than fine clots*⁶⁰⁻⁶²



Fine clots have more fibers to break down
*There is conflicting evidence of this behavior, which supports the need for interdisciplinary studies (e.g., modeling)^{15, 64}

Does tPA molecule to fiber ratio or tPA concentration govern rate of lysis?²⁵



Modeling has shown the ratio is the dominant driver rather than the concentration. This relates to how tPA in the clinic is delivered to patients in a fixed concentration based on body weight, rather than considering personal concentrations of important factors, such as fibrinogen.

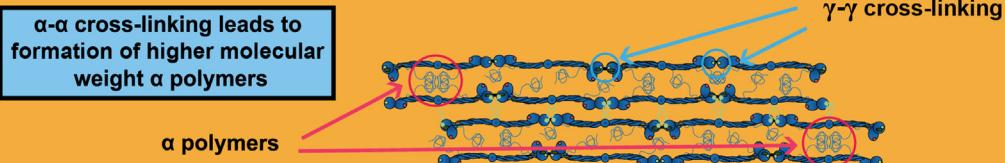
tPA: tissue plasminogen activator

Role of FXIIIa cross-linking^{40, 46, 65-72}

FXIII, also known as fibrin stabilizing factor, is a blood protein that cross-links fibrin

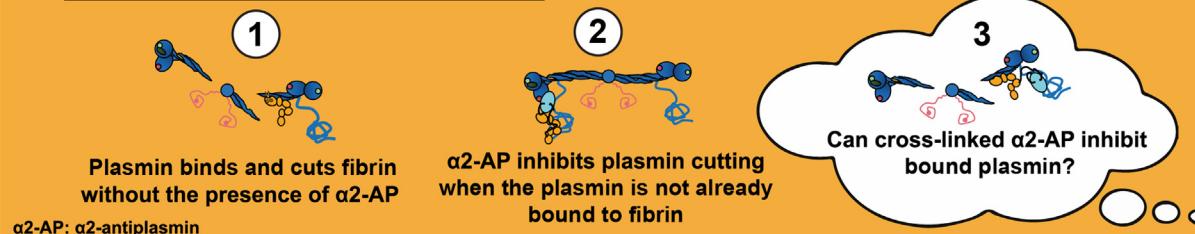
Permeability

α - α and γ - γ cross-linking decreases the space between fibers and can limit permeability



α 2-AP and resistance to lysis

FXIIIa prevents α 2-AP from being expelled from the network which makes the clot resistant to lysis. Below, we look at three scenarios with α 2-AP and plasmin when there is FXIIIa cross-linking.



Clot retraction

FXIIIa cross-linking and resulting clot retraction happens quickly and plays a large role in lysis inhibition

RBC retention

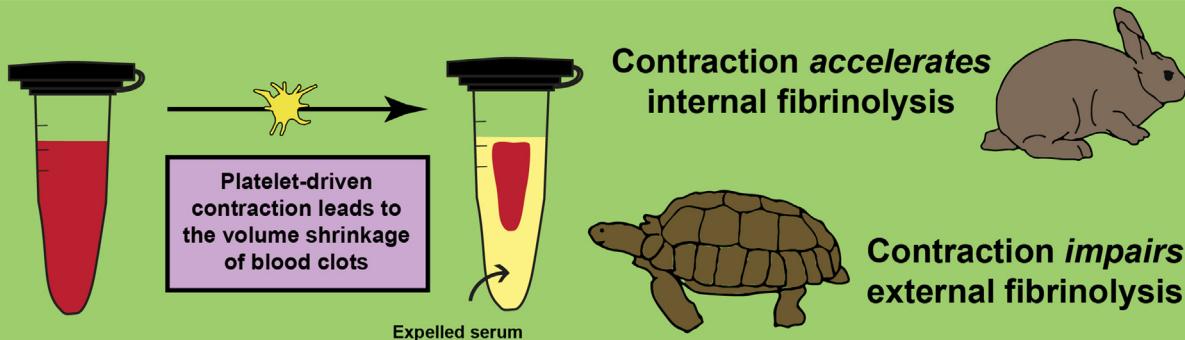
FXIIIa cross-linking aids in RBC retention, further limiting lysis

D-dimer production

D-dimers result from γ - γ cross-linking by FXIIIa.

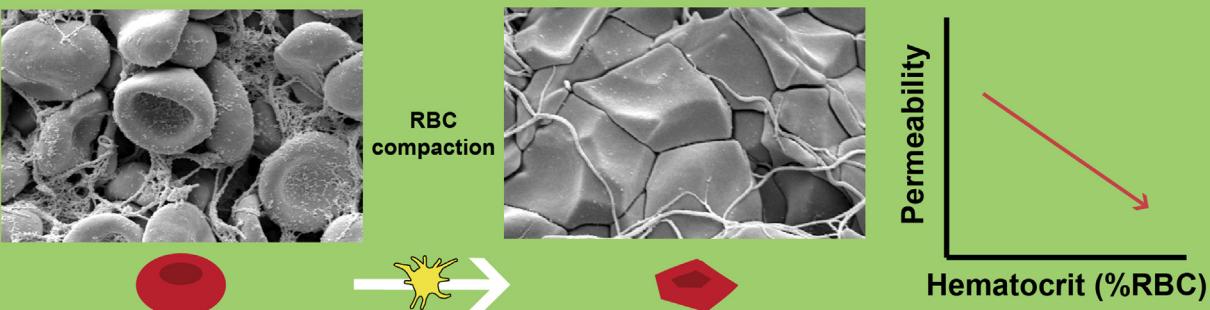
Influence of platelets on fibrinolysis⁷³⁻⁸⁷

Platelet-activated clot contraction^{63, 73-83}



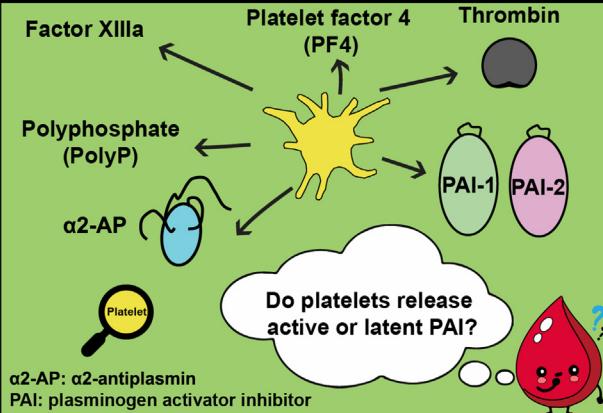
During clot contraction, platelets pull fibrin fibers together.
Clot contraction contradictorily modulates internal and external fibrinolysis.

Contraction reduces clot permeability⁸⁴

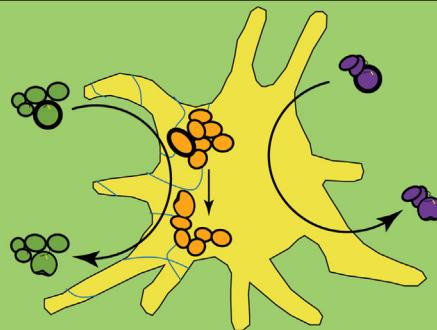


Red blood cells become densely packed in a tessellated polyhedral network during contraction, reducing the permeability of the clot and limiting lytic agent diffusion/perfusion

Platelets release fibrinolytic factors⁸⁵⁻⁸⁷



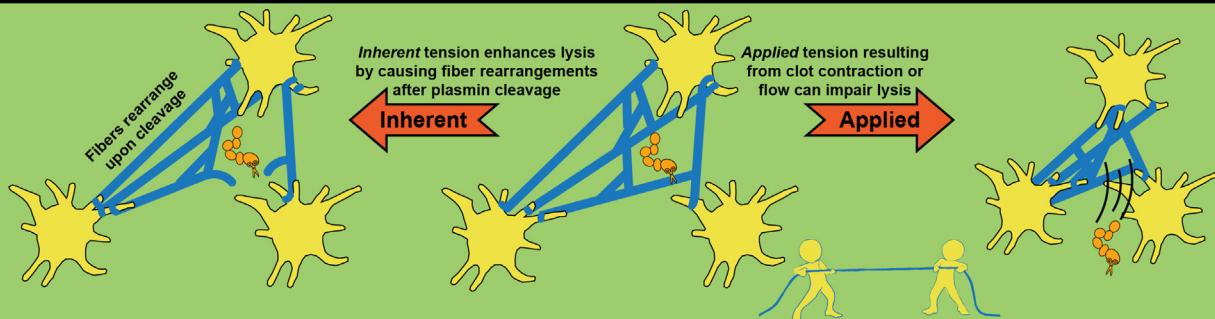
Platelets as activation sites^{74, 76, 88}



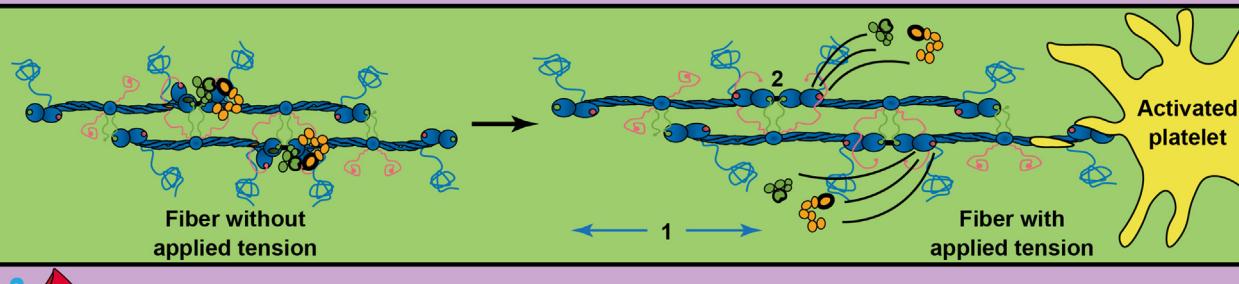
Platelet surfaces act as sites for activation of many fibrinolytic enzymes such as plasminogen and uPA

Platelets and fibrin-tension⁸⁹⁻⁹⁵

Platelets impact fiber tension and influence lysis

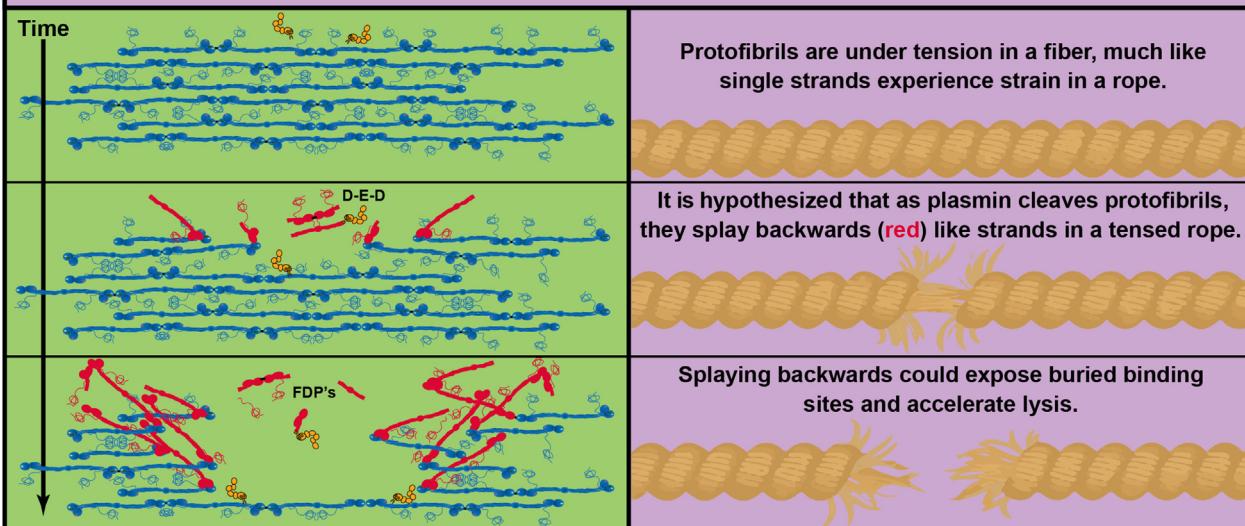


Fibers experience inherent tension upon polymerization and rearrange as select fibers are lysed (left), while platelets apply tension to a fibrin network by pulling and contracting a clot (right). Network shrinkage due to fiber rearrangements expels fibrinolytic molecules.



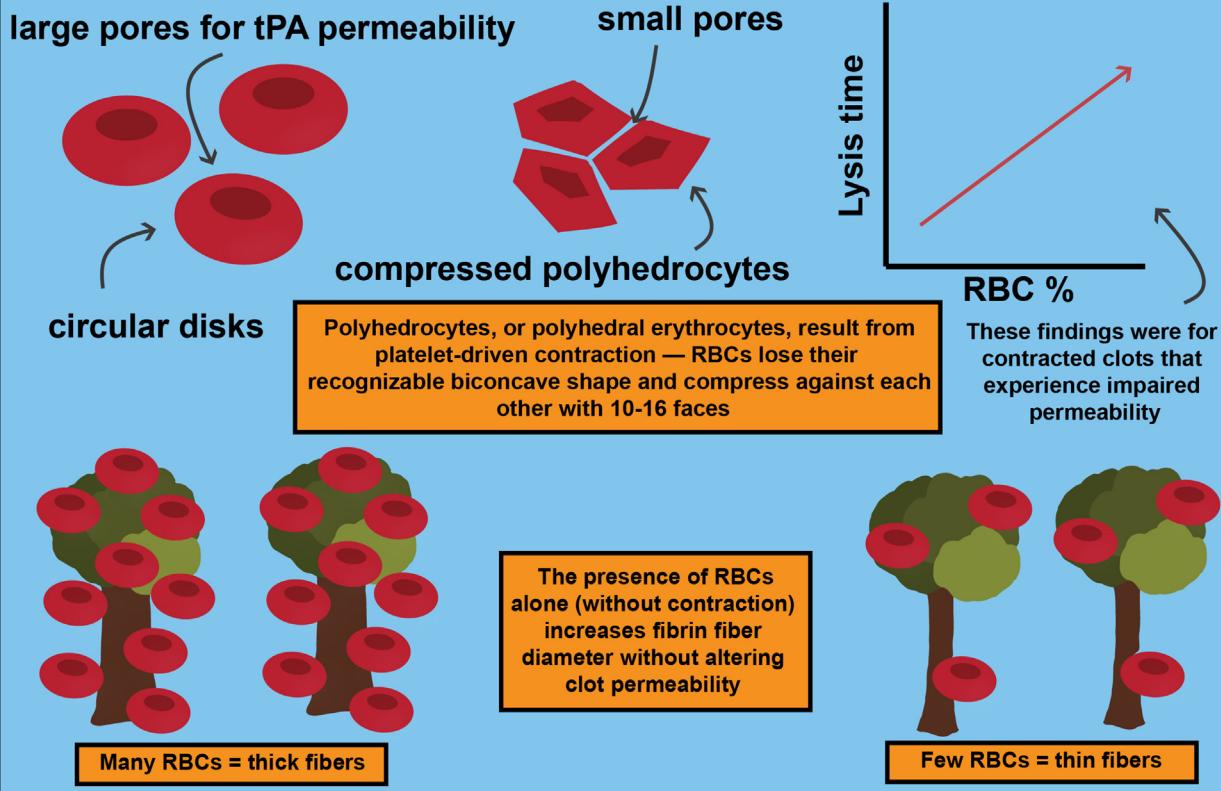
"Does molecular tension cause (1) elongation and/or (2) conformational change of D region that blocks plasminogen/tPA binding site?"

Does tension pull fibers apart during digestion?⁷

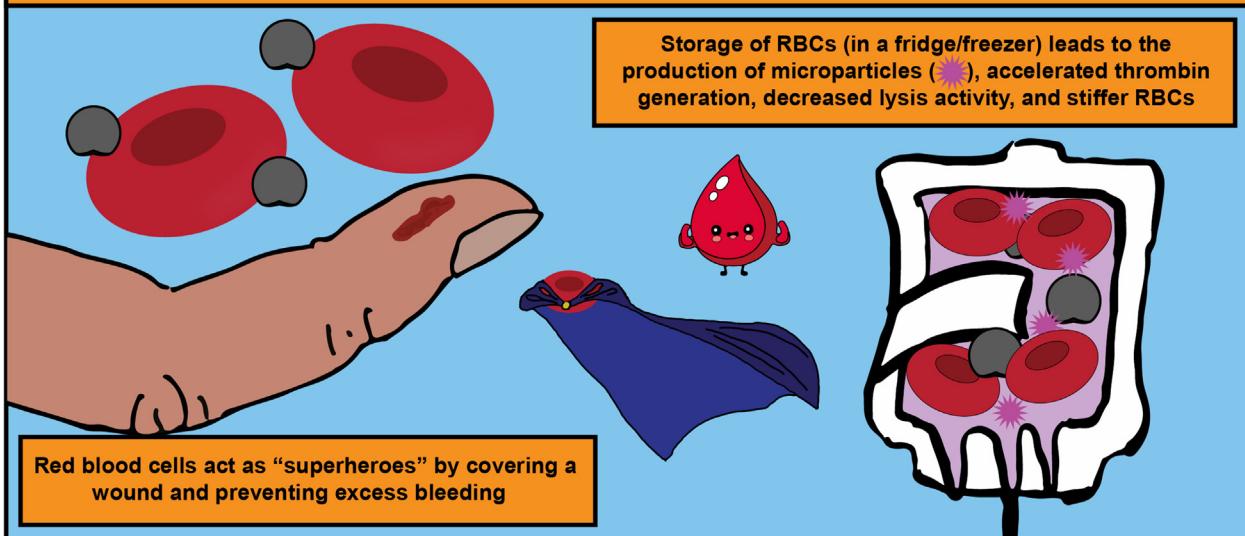


Influence of red blood cells on fibrinolysis⁹⁶⁻¹⁰⁰

Red blood cells (RBCs) take up space and compression limits lysis^{78, 96-100}

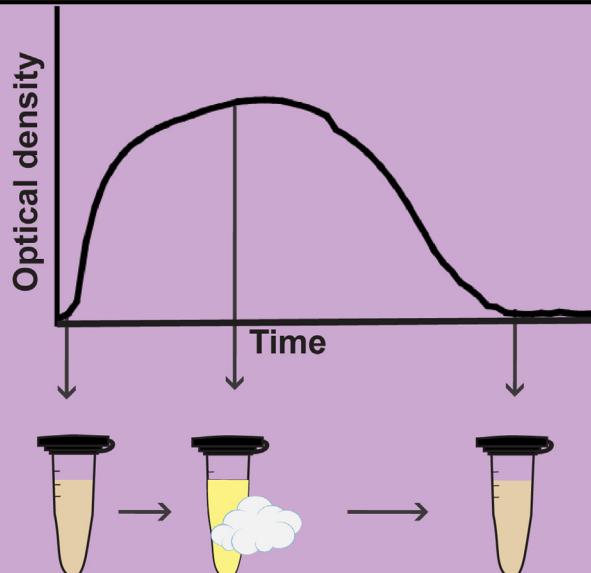


RBCs provide a procoagulant surface^{77, 101, 102}



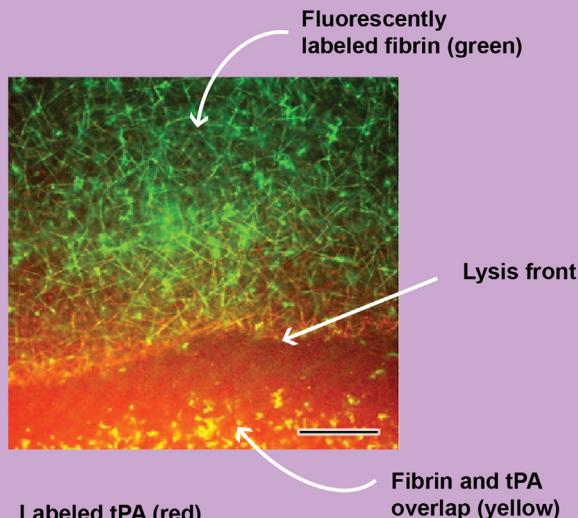
Laboratory techniques for analyzing fibrinolysis¹⁰³⁻¹¹⁹

Turbidity¹⁰⁵⁻¹⁰⁹



Transparent plasma becomes cloudy (or turbid) when a clot is formed. It turns back to transparent when lysed.

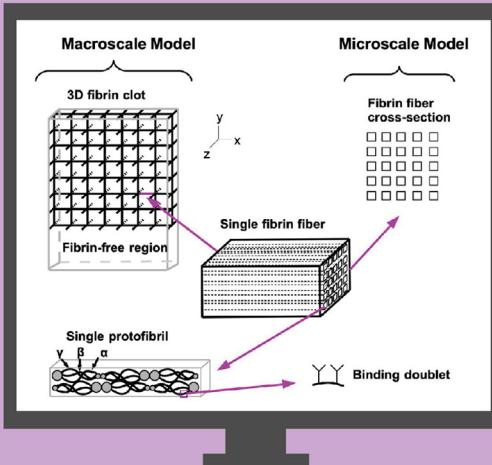
Fluorescent microscopy^{25, 28, 110-112}



Confocal microscopy allows researchers to statically or kinetically visualize the 3-dimensional fibrin network

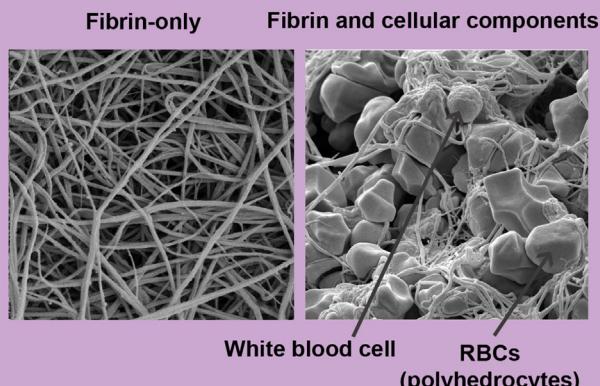
tPA: tissue plasminogen activator

Modeling^{113, 114}



Modeling can vary parameters in a way that cannot be done experimentally.

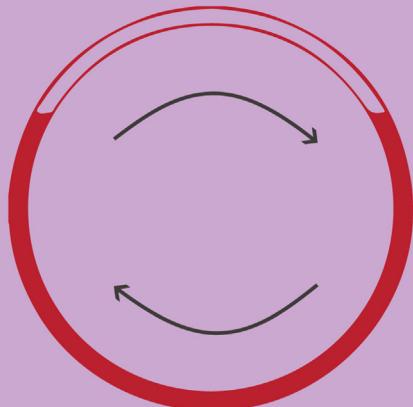
Scanning electron microscopy (SEM)^{111, 115}



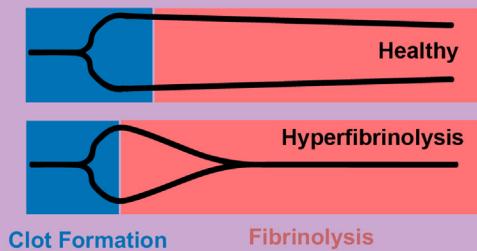
SEM clots are prepared through a number of dehydration steps. Lysis is initiated and immobilized before complete digestion.

Chandler loop¹¹⁶

A Chandler loop is an apparatus made of polymer tubes filled with circulating blood. This ex vivo model allows for testing of materials and lysis times with physiological blood flow.



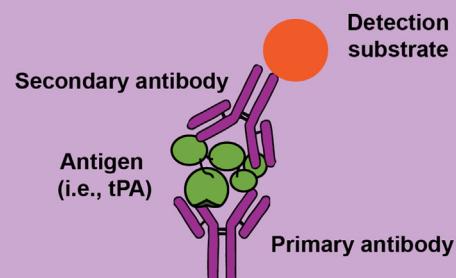
Viscoelastic mechanics¹¹⁷⁻¹¹⁹



Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) are used clinically to assess global hemostasis which includes assessment of fibrinolysis. Learn more in RPTH's illustrated review on viscoelastical testing!

Immunoassays

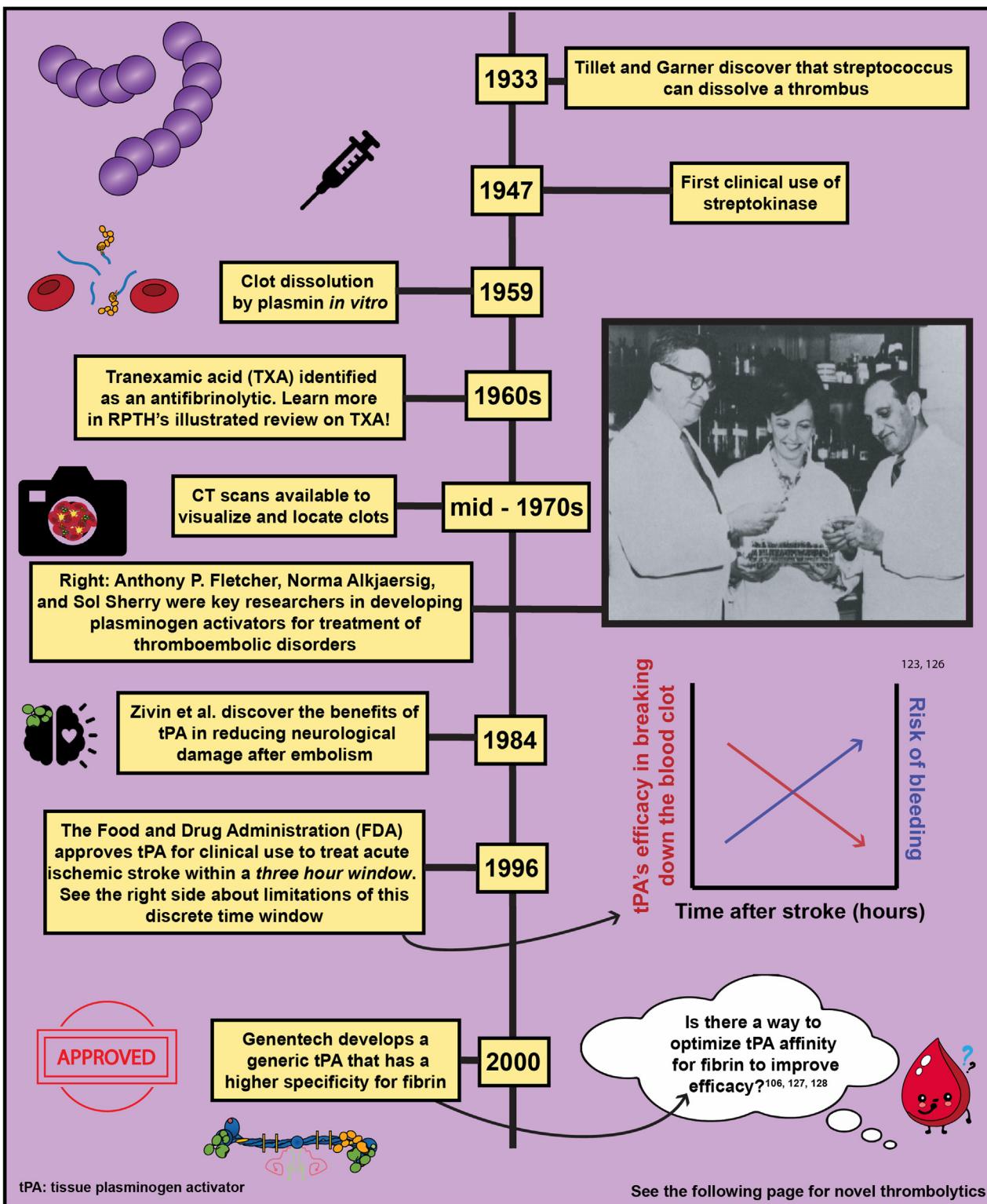
Immunoassays are used experimentally and clinically. They can be used to measure concentrations of clotting or fibrinolytic factors or the enzyme activity. An enzyme-linked immunosorbent assay (ELISA) is a common technique that uses antigen/antibody cocktails to quantify amount of proteins (i.e., tPA), protein-complexes (i.e., PAI-1 and tPA), or active vs latent states.



tPA: tissue plasminogen activator
uPA: urokinase plasminogen activator
PAI: plasminogen activator inhibitor

118-126

History of fibrinolytic development¹²⁰⁻¹²⁶

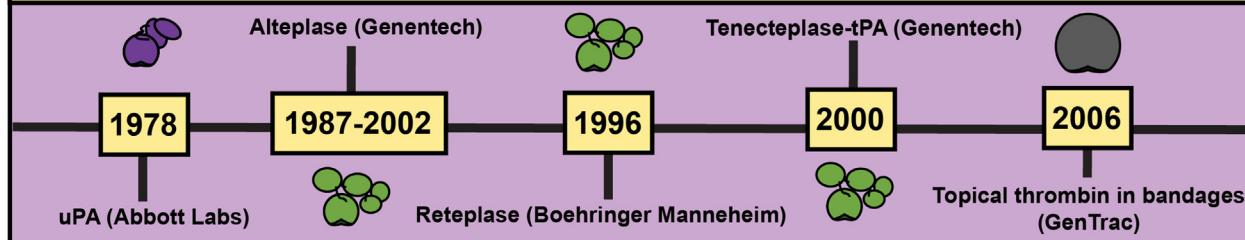


New translational perspectives on fibrinolysis

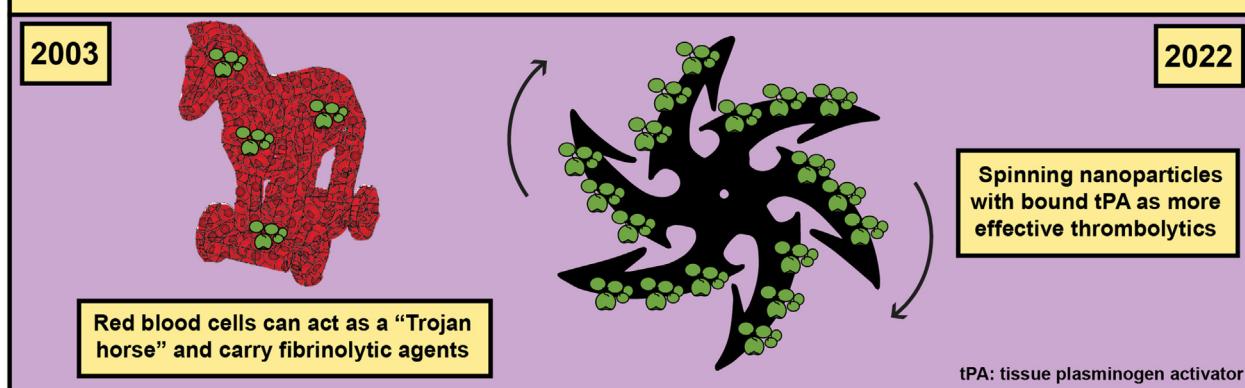
127-133

Recent advances in fibrinolytics have worked to improve delivery and efficacy. Check out RPTH's illustrated review that takes a closer look at two tPA thrombolytic agents used to treat ischemic stroke!

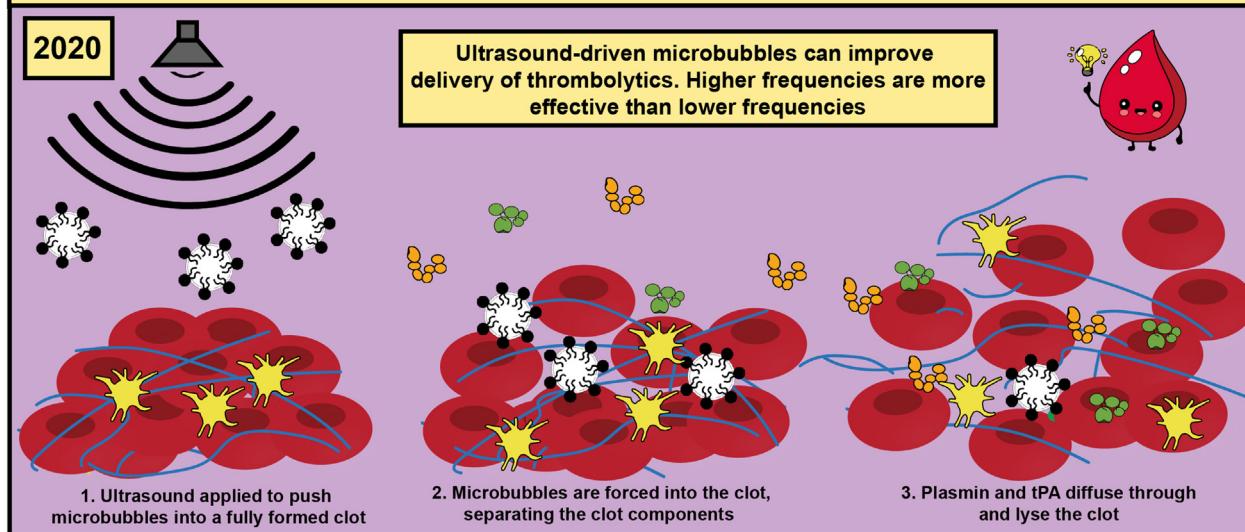
FDA approved thrombolytics/ fibrinolytics



RBCs and nanoparticles as delivery agents for thrombolytics¹²⁹⁻¹³²



Novel thrombolytics¹³³



ACKNOWLEDGMENTS

NIH R00HL148646-01 (V.T.), New Jersey Commission for Cancer Research COCR22PRF010

(R.R.), NIH T32 GM135141 (R.R.), NIH R15HL148842 (N.E.H.), and R15HL150666 (N.E.H.)

AUTHOR CONTRIBUTIONS

R.A.R., N.C.K., B.B., N.H., and V.T. contributed to creating all illustrations and writing the manuscript. All authors read and approved the final version of the paper.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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