Factor IX(a) inhibitors

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T.U.M.S

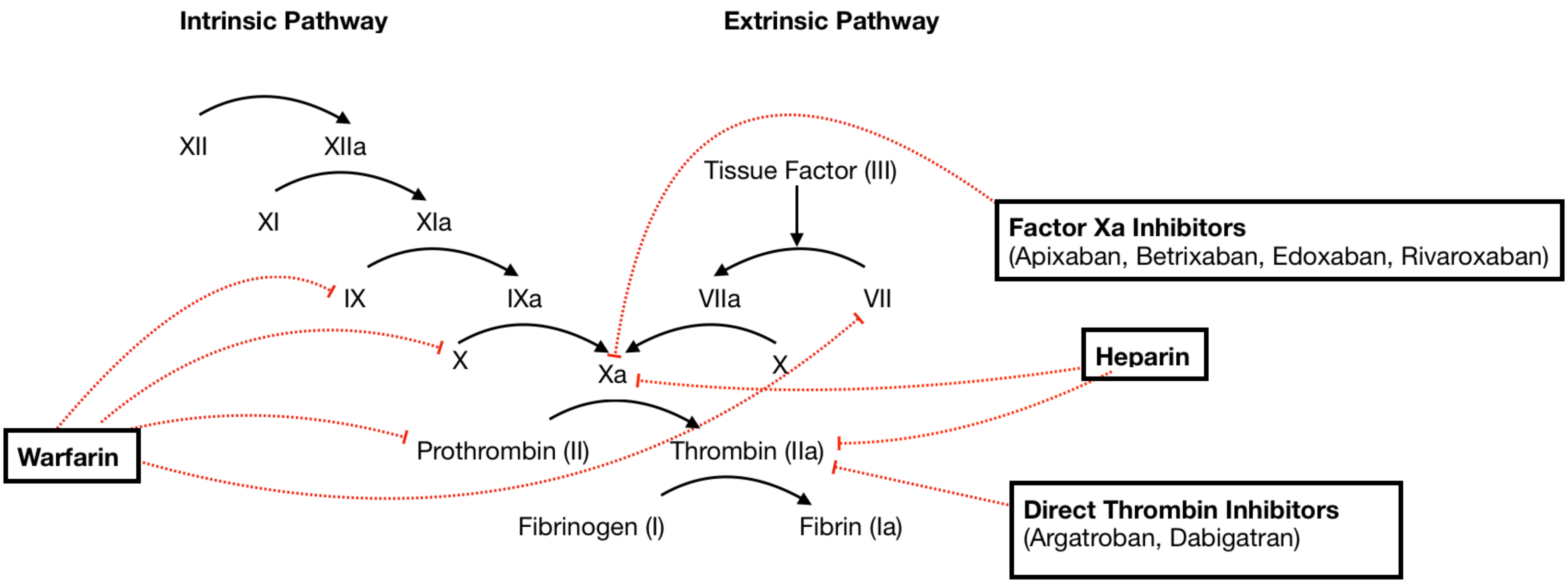
Introduction

- Thrombosis or pathological blood clotting is the leading cause of death globally
- Responsible for 25% of death cases worldwide
- Venous thrombosis (VT) and arterial thrombosis (AT)
- VT or "the red thrombi" as the blood clot is mainly constituted of fibrin i.e., the product of the coagulation process entrapping RBCs and, to a lesser extent, platelets
- AT or "the white thrombi" as the blood clot is mainly contributed to by platelets and contains less fibrin and fewer RBCs. In fact, the blood clot in AT conditions is platelet-rich

Antithrombotic agents

- Fibrinolytic agents, antiplatelet agents, and anticoagulant agents
- Four classes of anticoagulants
  - Heparins (unfractionated heparins, low molecular weight heparins, and fondaparinux)
  - Warfarin
  - Direct thrombin inhibitors such as bivalirudin, dabigatran etexilate, as well as argatroban
  - Direct factor Xa inhibitors such as rivaroxaban, apixaban, edoxaban, and betrixaban

- All clinically used anticoagulants are linked to an increased risk of bleeding because they target coagulation factors in the common coagulation i.e., thrombin and/or FXa which are actively involved in the formation of the crosslinked fibrin that is needed to seal the site of injury
- Because of this risk, the anticoagulants are underused by clinicians, particularly in the elderly.
- In the time range of 2008 – 2014, a study reported that about 40% of atrial fibrillation patients, who are at high risk for stroke, failed to receive anticoagulation therapy because of bleeding concerns



Intrinsic Pathway
Extrinsic Pathway
XII
XIIa
XI
XIa
IX
IXa
X
Xa
Tissue Factor (III)
VIIa
VII
X
Prothrombin (II)
Thrombin (IIa)
Fibrinogen (I)
Fibrin (Ia)
Warfarin
Factor Xa Inhibitors
(Apixaban, Betrixaban, Edoxaban, Rivaroxaban)
Heparin
Direct Thrombin Inhibitors
(Argatroban, Dabigatran)

- There are several novel anticoagulant agents under development targeting different components of the coagulation cascade
- These agents are claimed to treat and/or prevent thrombosis with little-to-none effect on hemostasis.
- The claimed components to be targeted include factor IXa, factor XIa, factor XIIa, and factor XIIIa

Structure and function of human FIX(a): The promise of a new anticoagulant target

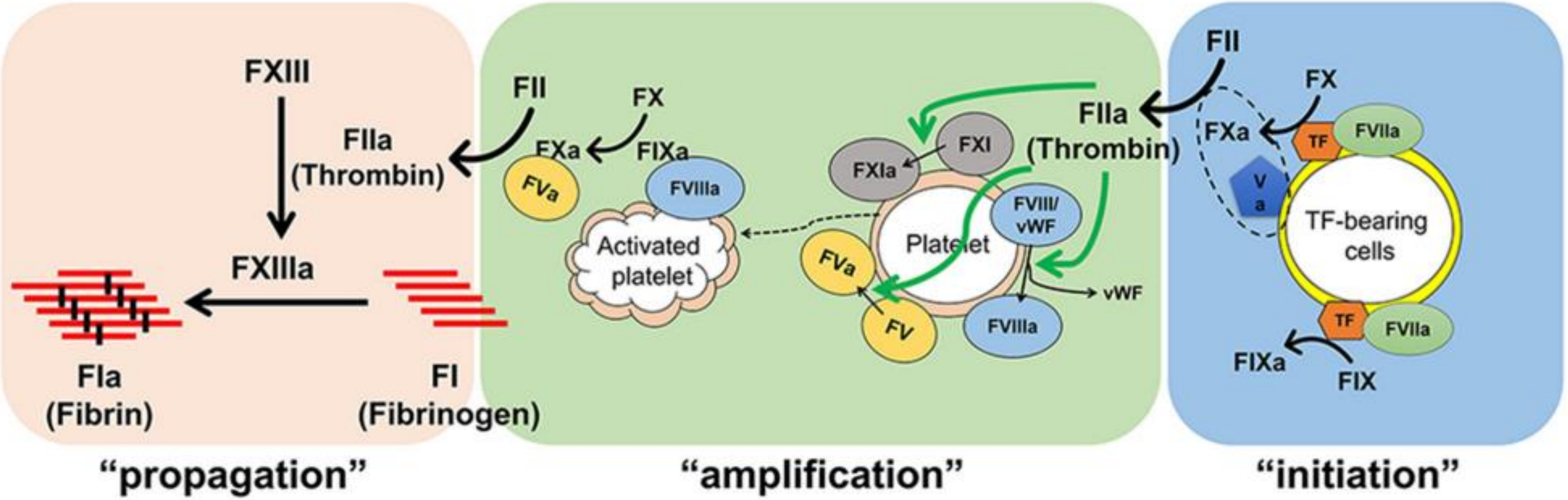
- FIX is a vitamin K-dependent protein that is biosynthesized by hepatocytes as a precursor of a serine protease, FIXa
- Researchers investigated the thrombogenicity of purified thrombin, FXa, and FIXa in the Wessler model of VT:
  - FIXa was found to be about 7-fold and 60-fold more thrombogenic than FXa and thrombin, respectively.
  - Likewise, inhibition of FIXa-induced VT needed heparin doses that were 2- to 4-fold lower than those required to inhibit clot formation in FXa-induced or thrombin-induced VT
- The loss of the intrinsic pathway was found to protect mice from polyphosphate-induced pulmonary embolism

- Another piece of evidence that supports FIXa as a viable target to design and develop new anticoagulant therapy is related to the manifestations of FIX deficiency in patients and carriers of hemophilia B.
  - The manifestation is highly dependent on the plasma FIX level.
  - The tendency to bleed from small wounds and during surgery decreases as FIXa levels increase in patients with mild hemophilia B (>5% FIX activity) and hemophilia B carriers (median FIX activity, 60%)
  - Mild FIX deficiency also did not prolong the aPTT or require prophylaxis to prevent bleeding during minor procedures, and in epidemiological studies, mild deficiency was found to be associated with fewer cardiovascular events

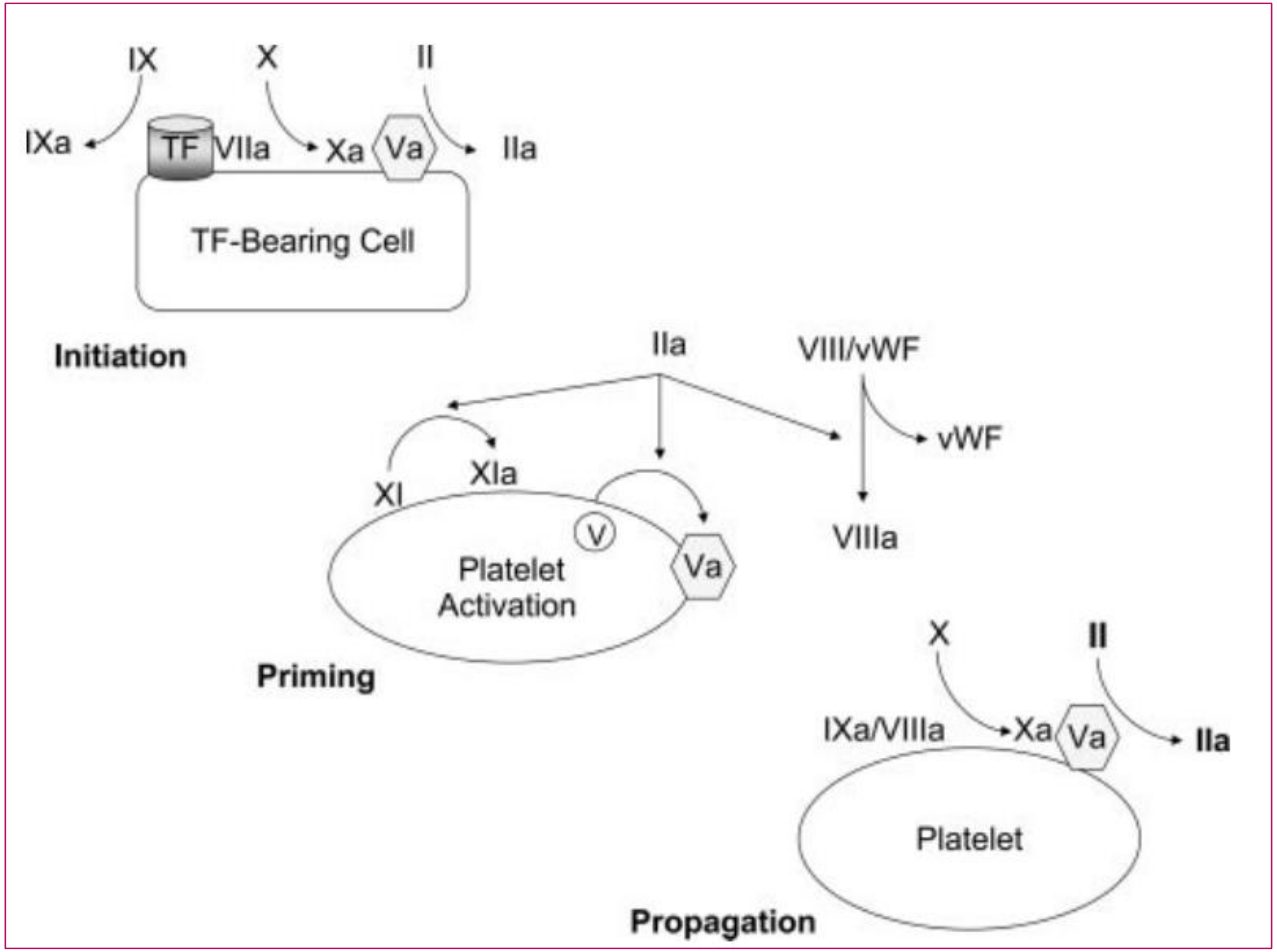
- Interestingly, in patients with moderate hemophilia B (1%–5% FIX activity), bleeding occurs only with trauma or surgery.
- Nevertheless, spontaneous bleeding occurs in patients with severe hemophilia (<1% FIX activity)
- It has been found that about 50-99% decrease in levels of FIX as that observed in the carriers of hemophilia type B or in mild or moderate hemophilia B is linked to a reduced risk of thrombosis with non-dangerous levels of bleeding

Why Factor IX inhibitors?

- Upstream target in the coagulation cascade
  - Factor IX belongs to the intrinsic pathway, so its inhibition does not completely block coagulation
- Extrinsic pathway remains active
  - The tissue factor–Factor VII pathway can still initiate clot formation during bleeding
- Partial reduction of thrombin generation
  - Thrombin production is reduced but not fully suppressed, preserving hemostasis
- Role in amplification, not initiation
  - Factor IX mainly amplifies clot formation rather than starting it.
- More selective anticoagulant effect
  - Leads to effective thrombosis prevention with a lower risk of bleeding

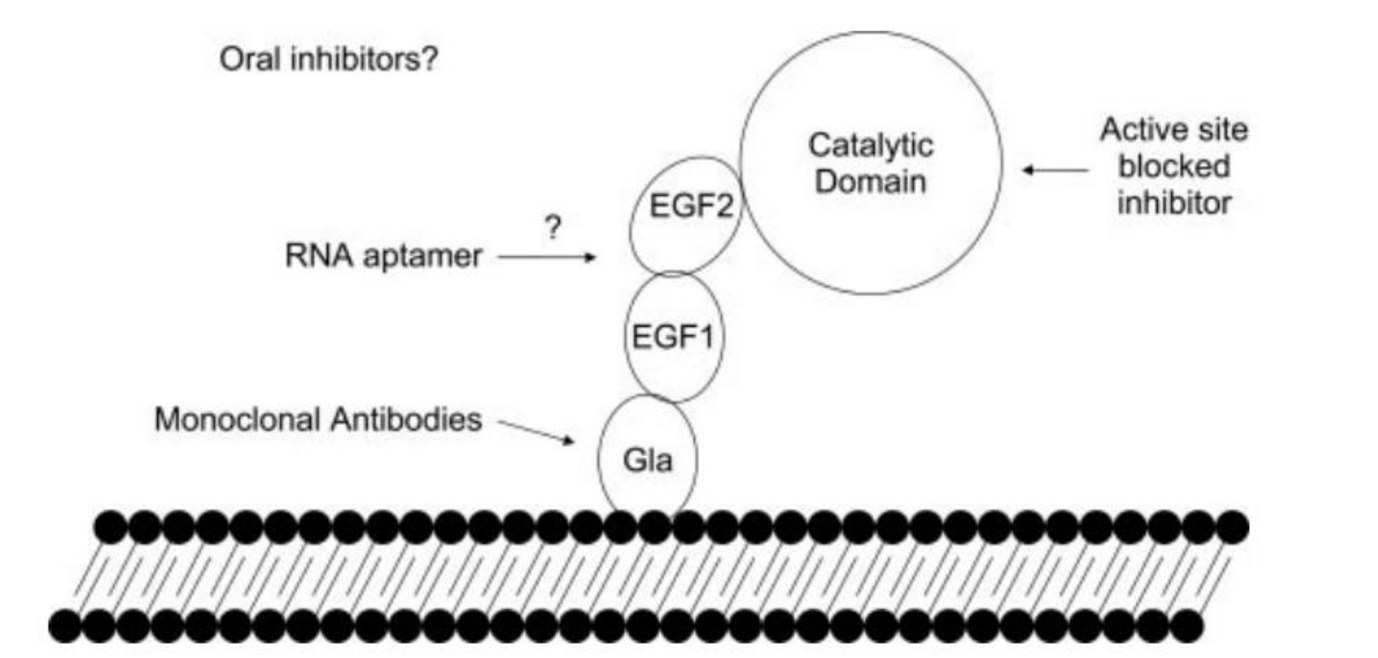


FXIII
FIIa
(Thrombin)
FXIIIa
FIa
(Fibrin)
FI
(Fibrinogen)
FII
FX
FXa
FIXa
FVa
FVIIIa
Activated platelet
FXI
FXIa
FVIII/
vWF
Platelet
FVa
FV
FVIIIa
vWF
FIIa
(Thrombin)
FII
FX
FXa
TF
FVIIa
V
a
TF-bearing cells
TF
FVIIa
FIXa
FIX
"propagation"
"amplification"
"initiation"


IX
X
II
IXa
TF
VIIa
Xa
Va
IIa
TF-Bearing Cell
Initiation
IIa
VIII/vWF
vWF
XI
XIa
V
Va
VIIIa
Platelet Activation
Priming
X
II
IXa/VIIIa
Xa
Va
IIa
Platelet
Propagation

Factor IXa Inhibitors

No currently approved direct FIX inhibitor for thrombosis



Active Site-Blocked Competitive Antagonists

- The earliest investigation of factor IXa inhibitors used the competitive antagonist IXai.
- The active site of IXa is blocked by incubation with dansyl-glutamyl-glycyl-arginyl-chloromethylketone, yielding a protein without functional coagulant activity.
- Thus, IXai functions as a competitive inhibitor of factor IXa binding to platelets
- In several animal models, IXai functioned as an effective anticoagulant with limited bleeding complications
  - Canine model of coronary thrombosis , cardiopulmonary bypass
  - Rabbit model of synthetic patch angioplasty, arterial thrombosis
  - Rat model of stroke

Oral inhibitors

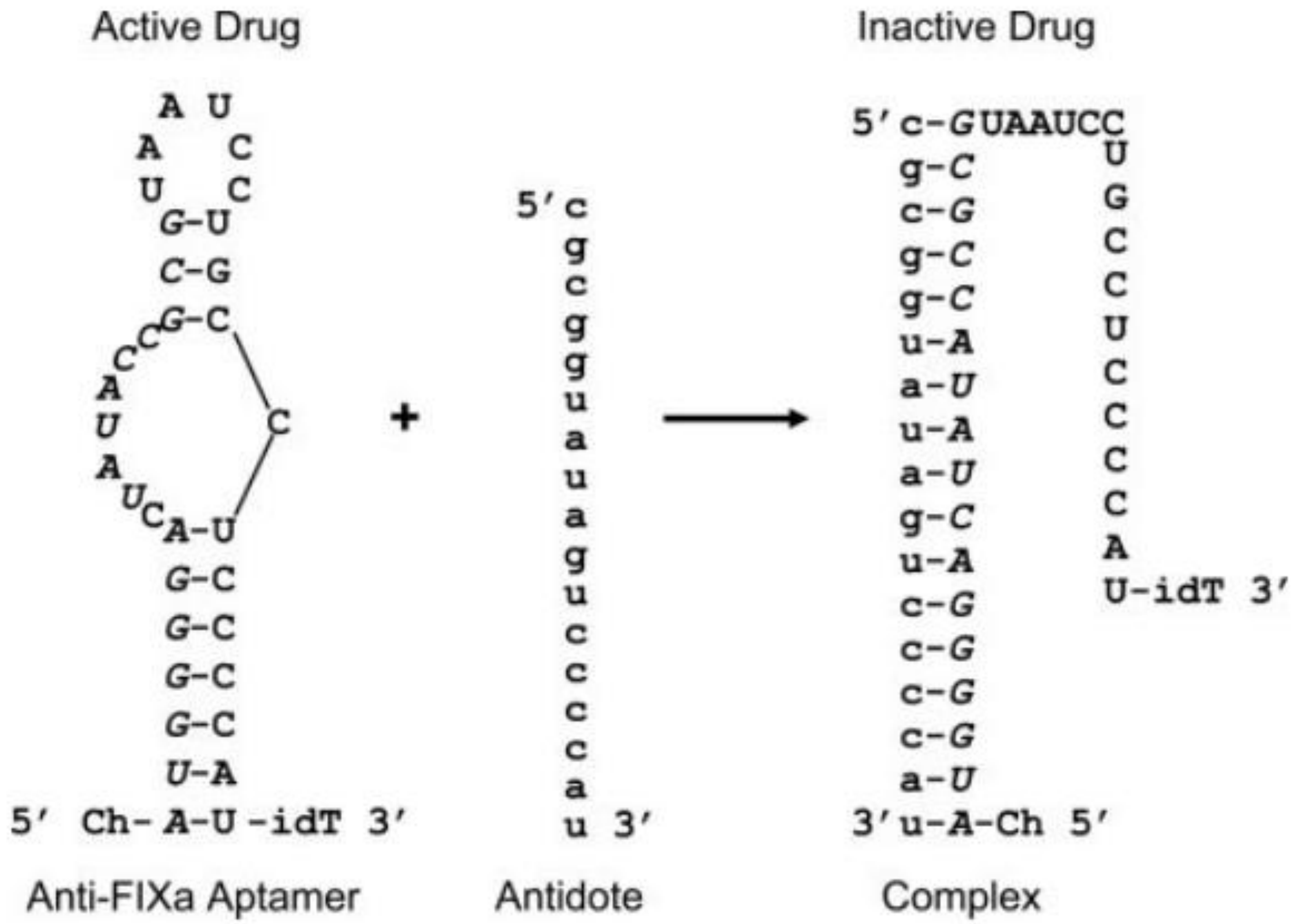
- TTP889 is a small molecule and orally administered inhibitor of FIXa.
- The structure of TTP889 is not published and its mechanism appears to be the inhibition of the ability of FIXa to bind to FVIIIa, which subsequently, inhibits the generation of a functional tenase complex
- A once daily dose of TTP889 (300 mg) for extended thromboprophylaxis in N= 261 patients who had undergone surgery for hip fracture, resulted in a limited difference in the primary efficacy outcome of venous thromboembolism in comparison with the placebo patients
- There were also no major bleeding events and only two non-major bleeding events upon the administration of TTP889.
- Due to the lack of efficacy, development of TTP889 for this purpose appears to be had been discontinued.

Monoclonal Antibodies

- Several antibodies against epitopes of factor IX have been developed.
- SB 249417
  - A chimeric monoclonal antibody, directed against the human factor IX Gla domain
  - In a rat model of arterial thrombosis, the antibody achieved significant reductions in thrombus formation with modest prolongation of the aPTT
  - In a rat model of stroke, reduced infarct volume and was associated with reduced neurological deficits in animals treated with this antibody compared with animals treated with tissue plasminogen activator.
  - A phase I clinical trial with SB 249417 has been completed.

RNA Aptamers

- Aptamers are oligonucleotides that adopt a specific three-dimensional shape that permits its specific recognition of a specific protein
- They are typically produced in an in vitro process known as Systemic Evolution of Ligands by EXponential enrichment (SELEX)
- The complementary binding between the aptamer and its protein target typically happens via a series of nucleotide– protein interactions such as H-bonds, ionic bonds, and hydrophobic ones
- To target FIX, an RNA aptamer 9.3t was isolated using the SELEX technology and to prolong its circulating half-life in vivo polyethylglycerol or cholesterol was linked to the aptamer 5’-end.
- A complimentary RNA antidotes (the best to be chosen was RB007 or anivamersen) were also engineered against the conjugated RNA aptamers (the best was later termed as RB006 or REG1)


Active Drug
Inactive Drug
5′ Ch- A-U -idT 3′
5′c
u 3′
5′c-GUAAUCC
U-idT 3′
3′u-A-Ch 5′
Anti-FIXa Aptamer
Antidote
Complex

- The aptamer was reported to bind with high affinity and specificity to FIX/FIXa, but not to FVII, FX, FXI, or protein C
- Mechanistically, the RNA aptamer was predicted to bind to the EGF domains, prolonged the APTT of human plasma, and its action was dose-dependently reversed by RB007
- Several in vivo studies have validated the above findings in pigs, murine model of arterial injury and murine tail bleeding assay , porcine model of cardiopulmonary bypass , and baboon model of cardiopulmonary bypass

- Phase II RADAR study
  - Patients (N=64) with non- ST- segment elevation acute coronary syndrome with planned early cardiac catheterization via femoral access were randomized to heparin or REG1 with RB007 reversal
  - At least 50% reversal of REG1 was needed to prevent bleeding after sheath removal
- Phase III REGULATEPCI percutaneous coronary intervention trial,
  - Patients (N=3232) undergoing percutaneous coronary intervention for non-ST-segment elevation acute coronary syndrome were randomized to bivalirudin or REG1 with 80% RB007 reversal.
  - The study was stopped early after the report of severe allergic reactions

Challenges and Limitations

- The new drugs must exhibit either improved efficacy and safety or at least similar efficacy, but improved safety compared with the current drugs, particularly with respect to the bleeding complication risk
- Establishing the clinical value of any FXI(a)-targeting drug would require expensive head-to-head studies in which their efficacy, safety, tolerability, pharmacodynamic, and pharmacokinetics as anticoagulants are to be compared with the direct orally active site inhibitors of thrombin or FXa.
- The studies will be expensive due to the need to involve large numbers of diverse patients with various co-morbidities.

Potential Niche Applications:

- To avoid such obstacle, specific indications need to be identified such that the novel FIX(a)-targeting agents can be compared with the current anticoagulants that are known to have an issue or even fail to deliver an optimal clinical outcome, especially without a clinically significant major bleeding or other side effects.
- Prevent and/or treat:
  - Stroke prevention in atrial fibrillation patients
  - Cardiovascular events in patients with chronic kidney disease or those undergoing hemodialysis
  - VT in patients who have an increased risk of recurrent thrombosis upon anticoagulant discontinuation
  - Clotting in extracorporeal membrane oxygenation circuits
  - Thromboembolic events in patients with cardiac devices
  - Stent thrombosis in acute coronary syndrome patients who need anticoagulant therapy in addition to antiplatelet therapy
- In all of the above proposed indications, the current state-of-the-art anticoagulants have serious problems.

Role of Nanoparticles in Thrombosis Therapy

- The existing thrombus treatment methods still encounter three problems
  - The physicochemical and pharmacokinetic defects of conventional antithrombotic drugs, including short half-life and narrow therapeutic window.
  - The low efficiency of thrombotic therapy
  - The occurrence of side effects such as bleeding
- Nanomedicine has received widespread attention since its introduction in the late 1990s.
- The superiorities of nanoparticles (NPs) for medical applications include reducing drug toxicity, prolonging half-life, and reducing side effects by modifying the properties of NPs while maintaining the biocompatibility of the particles

- In the field of oncology therapy, rational drug delivery systems based on NPs have shown promise for facilitating efficient administration of therapeutic drugs.
- There are similarities between thrombus and tumor in drug delivery.
- In the last decade, researchers have developed various nanocarriers, including inorganic particles such as mesoporous silica, metal NPs, and organic particles including micelles, liposomes, and polymers & natural blood cells
- By functionally modifying these nanocarriers, it is possible to achieve targeted delivery of thrombotic medicines

- For optimal drug delivery, the delivery system should be localized to the thrombus site and maintain drug enrichment to maximize thrombolytic efficiency, while avoiding unnecessary uptake by other tissues

- Therefore, drug delivery systems based on NPs with surface modification using targeted ligands for selective delivery of antithrombotic drugs to thrombus-specific sites may provide a more efficient solution.

Platelets

- Activated platelets are one of the key components of thrombus
- During thrombosis, platelets first attach to the injured vessel wall via their surface receptors, activating internal signaling pathways that secrete additional thrombus-stimulating factors and ultimately, accelerating the formation of biologically threatening fibrin networks
- So, targeting specific receptors on the activated platelet surface can avoid additional thrombogenic risks

- P-selectin
  - An adhesion receptor expressed on the surface of activated platelets and endothelial cells
  - Important role in arterial thrombus by forming large and stable platelet-leukocyte polymers
  - The main ligand of P-selectin is P-selectin glycoprotein ligand-1 (PSGL-1)
  - The binding of the leukocyte with P-selectin involves the Sialyl Lewis x (SLe x)
  - Fuco is a sulfated and fucosylated polysaccharide derived from brown algae, and its inclusion of the L-fucose moiety makes it a natural SLe x mimic
  - Fuco is expected to be used as a tool for drug-loaded NPs to recognize thrombus
  - Juenet et al. designed Fuco functionalized polymer NPs loaded with rtPA for the first time
  - A twofold increase in thrombolytic capacity in vitro and a more than tenfold increase in urokinase plasminogen activator (uPA) accumulation in thrombi in vivo

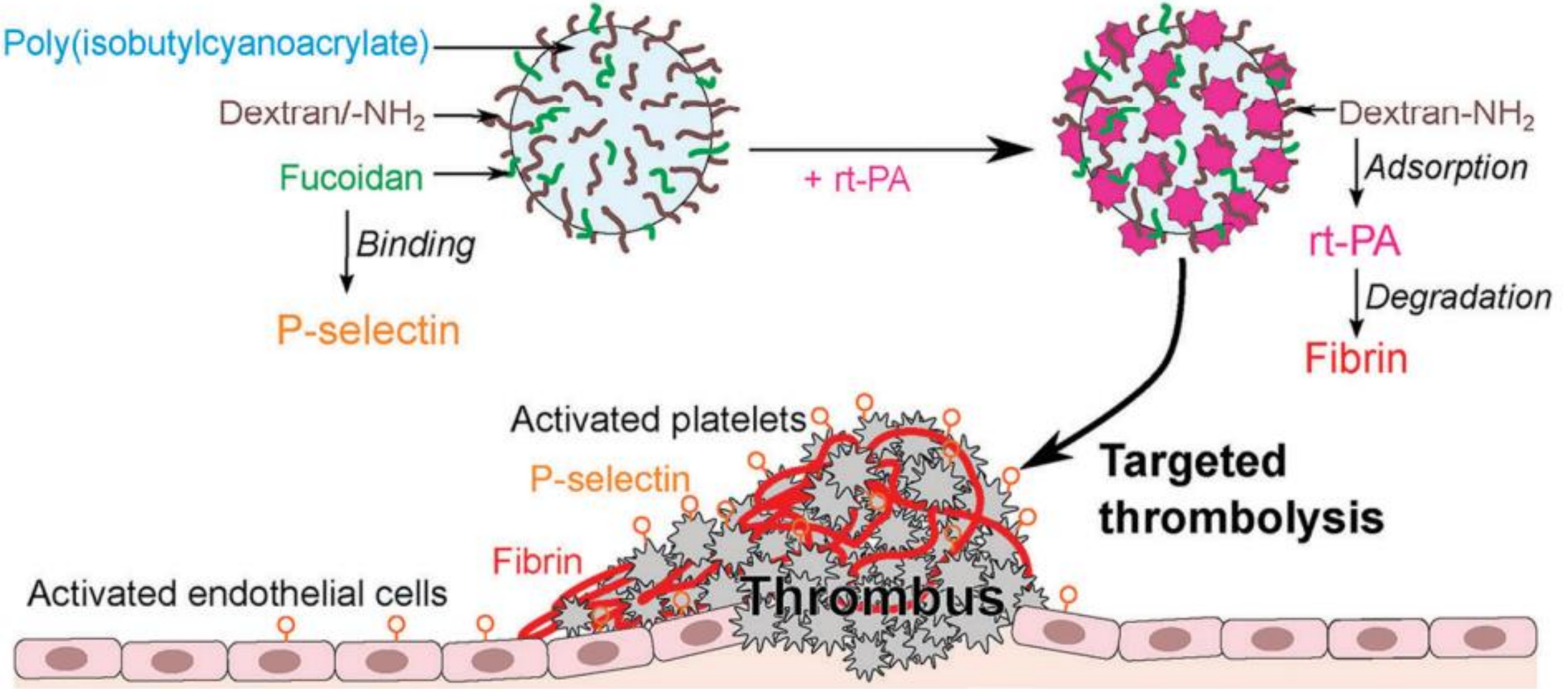
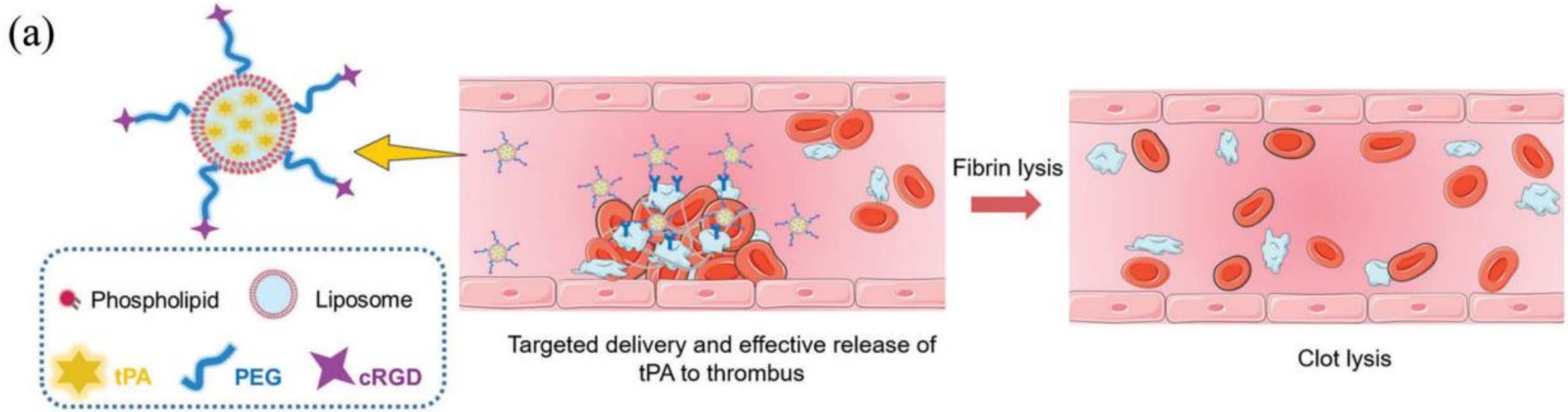


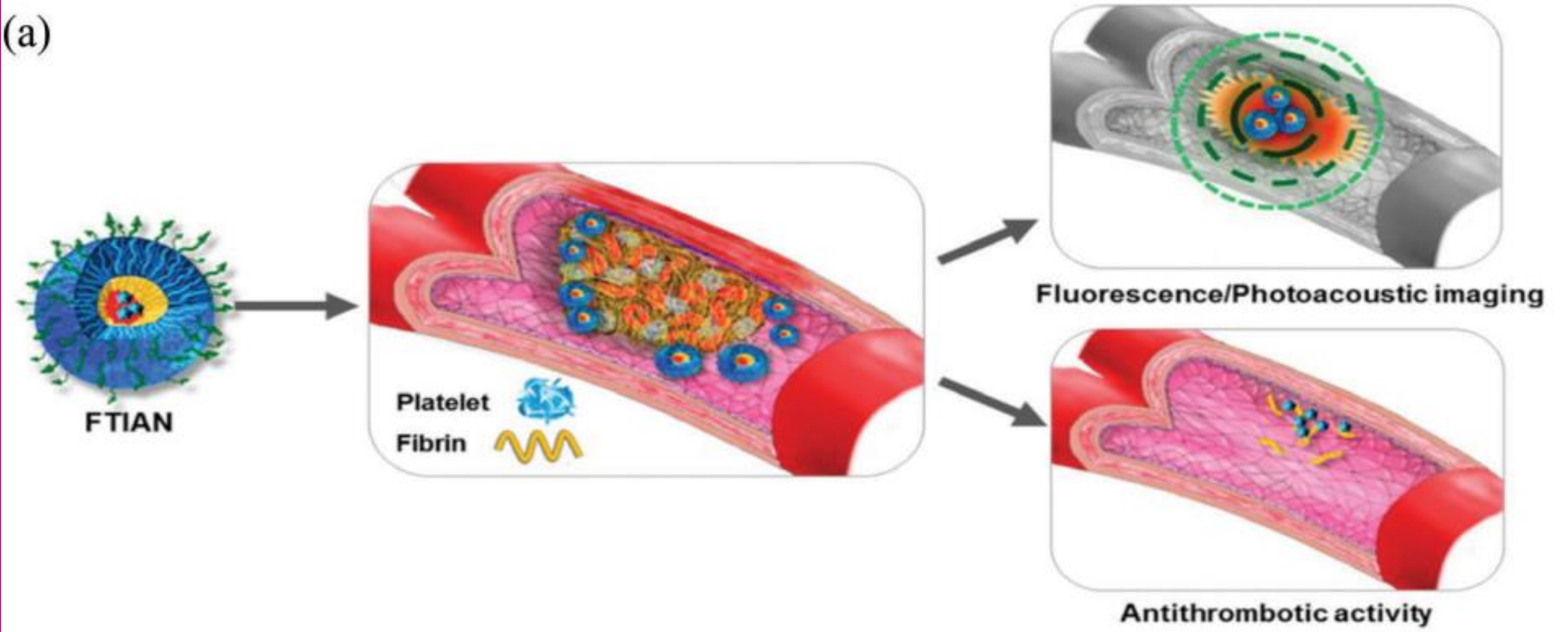
Figure 4. Polysaccharide-poly (isobutyl cyanoacrylate) NPs functionalized with Fuco to target P-selectin and promote the specific accumulation of loaded rt-PA in thrombi. Reproduced with permission.[78] Copyright 2018, Elsevier.

- GP IIb/IIIa
  - The most abundant membrane protein on the platelet surface.
  - It binds soluble ligands, including fibrinogen
  - The fibrinogen-derived peptide RGD binds specifically to GP IIb/IIIa
  - It has been approved that cyclic RGD has a stronger affinity for thrombus than linear structures; and therefore, cRGD has been widely used for thrombus molecular targeting in recent years
  - Huang et al. used PEG-encapsulated liposomes to encapsulate tPA.
  - The PEGylated liposomes encapsulated with cRGD could induce membrane fusion by high specificity binding to activated platelets resulting in the selective release of tPA at the clot site
  - In vitro clot lysis assay showed that more than half of tPA could be released from tPA-PEG-cRGD-lip at 75 min, leading to most clot lysis



(a)
Phospholipid
Liposome
tPA
PEG
cRGD
Targeted delivery and effective release of
tPA to thrombus
Fibrin lysis
Clot lysis

- Fibrin:
  - Fibrinogen is cleaved into fibrin by thrombin and coagulation factors during thrombosis
  - The pentapeptide cysteine–arginine–glutamic acid-lysine-alanine (CREKA), is known to be a clot-binding peptide that has good fibrin targeting ability
  - Kang et al. used CREKA to design perfluorohexane (PFH)-based artificial NPs, denoted FTIAN
  - FTIAN specifically targeted the obstructive thrombus and significantly enhanced the fluorescence/photoacoustic signal.
  - When loaded with the antiplatelet drug tirofiban, FTIAN remarkably suppressed thrombus formation



(a)
FTIAN
Platelet
Fibrin
Fluorescence/Photoacoustic imaging
Antithrombotic activity

Microenvironment-Responsive Technologies

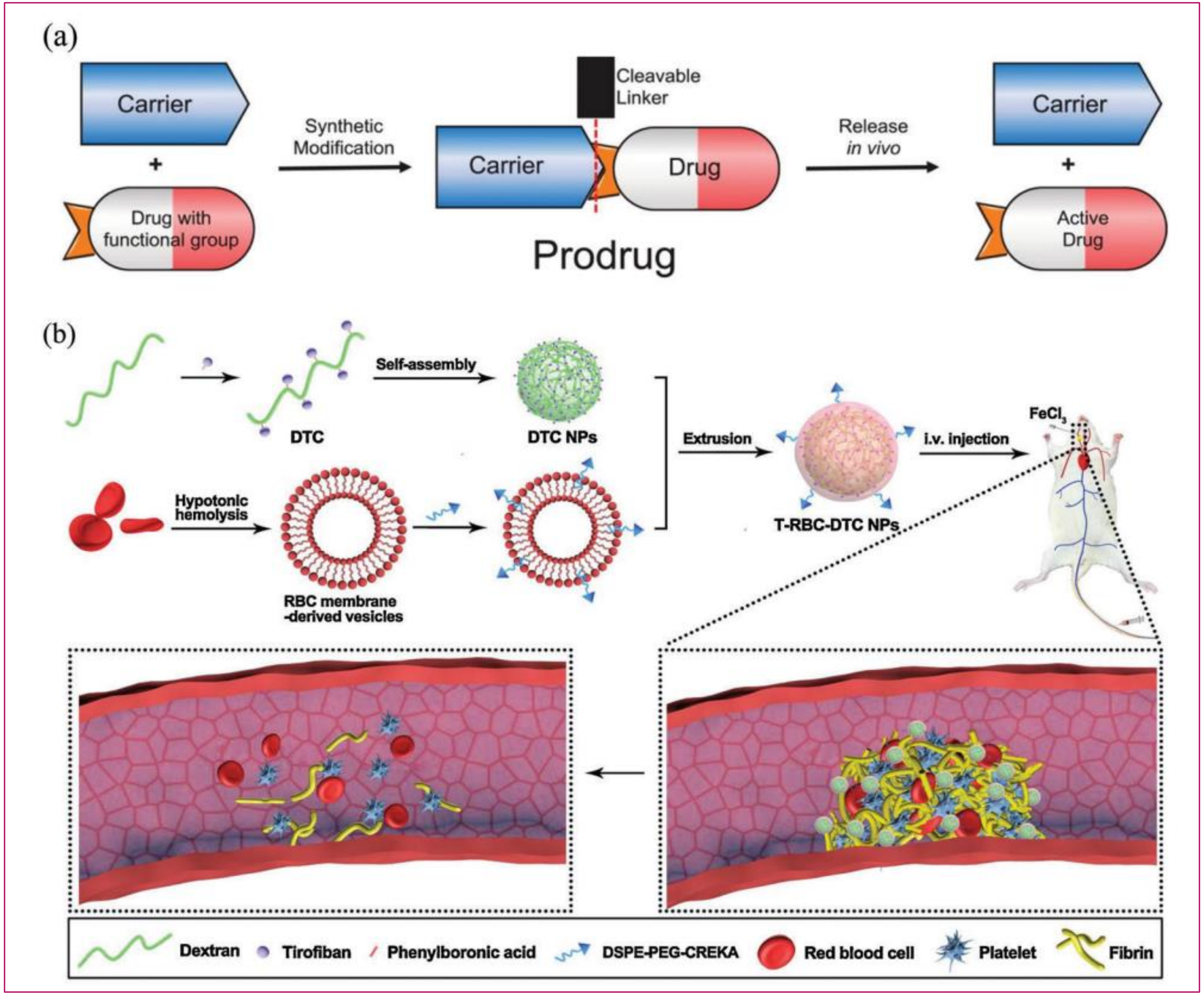
- Although researchers have developed a variety of nano-drug delivery modalities for thrombosis therapy, successful translation of these modalities to clinical applications remains challenging due to concerns about drug leakage during transport.

- Thrombus formation is accompanied by changes in the surrounding microenvironment, including an increase in H_2O_2, and a decrease in pH value

- These microenvironmental changes are not only products of thrombosis but can also influence thrombotic progression

H_2O_2

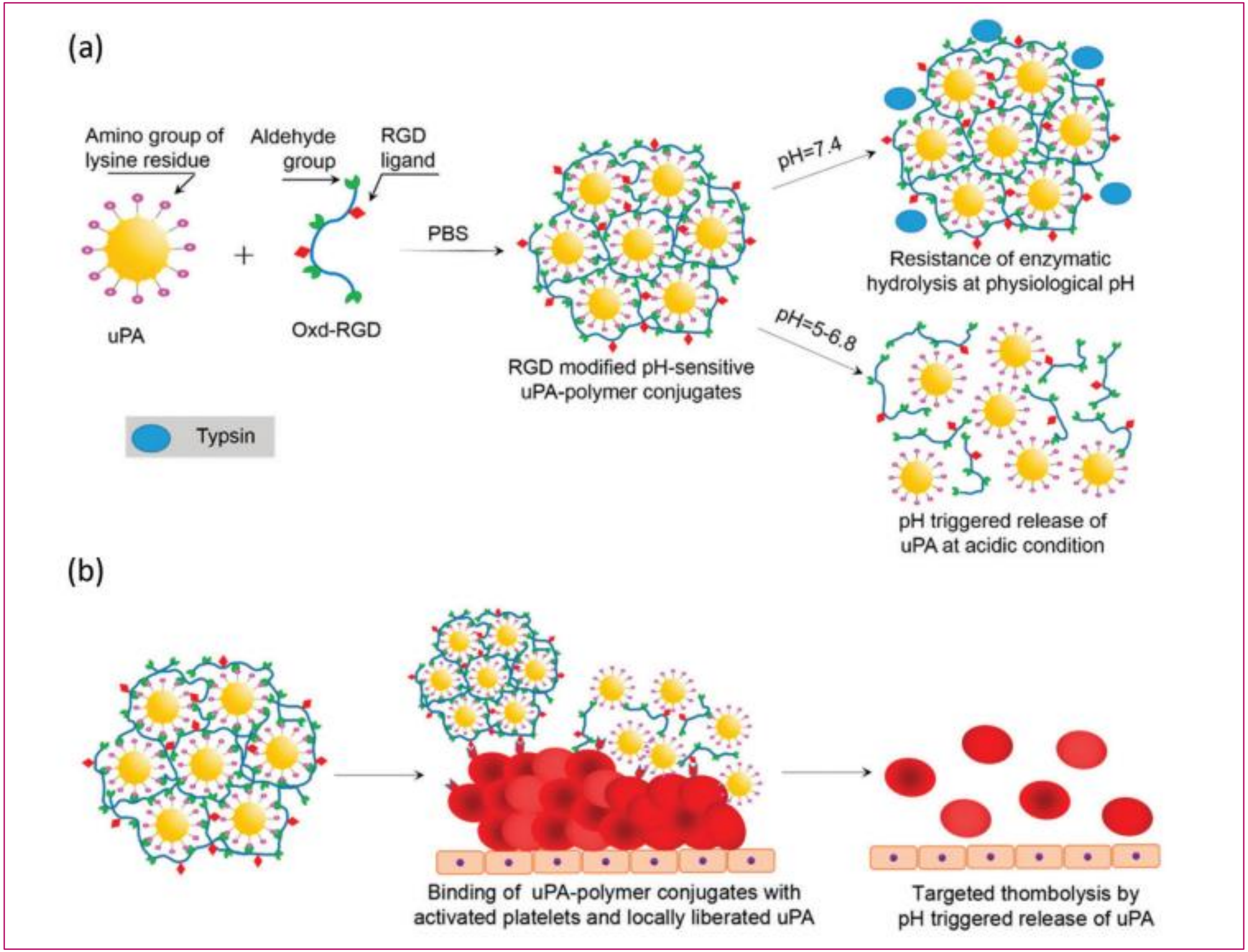
- Zhao et al. designed self-assembled dextran–tirofiban conjugate nanoparticles (DTC NPs) using phenylboronic acid ester bonds to deliver fibrinogen-dependent inhibition of platelet aggregation with the antiplatelet drug tirofiban.
- DTC NPs could be rapidly oxidatively cleaved by H_2O_2, thereby scavenging excess H_2O_2 during the reaction.
- In addition, the H_2O_2 -reactive junction inhibited drug leakage during circulation and provided controlled drug release at the thrombus site
- They showed that treatment with DTC NPs effectively inhibited thrombosis (14.9% thrombosis) compared to an equivalent amount of tirofiban (88.1% thrombosis level)



(a)
Carrier
+
Drug with functional group
Synthetic Modification
Cleavable Linker
Carrier
Drug
Prodrug
Release in vivo
Carrier
+
Active Drug
(b)
DTC
Self-assembly
DTC NPs
Hypotonic hemolysis
RBC membrane -derived vesicles
Extrusion
T-RBC-DTC NPs
i.v. injection
FeCl3
Dextran
Tirofiban
Phenylboronic acid
DSPE-PEG-CREKA
Red blood cell
Platelet
Fibrin

pH

- It has been reported that anaerobic glycolysis decreases pH at the thrombus site, resulting in an anaerobic acidic environment distinct from normal tissue
- Li et al. introduced a pH-sensitive imine linkage with a pH-sensitive bond in the drug delivery system and used this bond to link uPA to oxidized dextran
- The nanosystem was also modified with platelet-targeted RGD peptides.
- Hydrolysis of the imine bonds of the polymer in the weak acidic environment surrounding the thrombus released the uPA encapsulated therein


(a)
Amino group of lysine residue
Aldehyde group
RGD ligand
uPA
Oxd-RGD
PBS
RGD modified pH-sensitive uPA-polymer conjugates
pH=7.4
Resistance of enzymatic hydrolysis at physiological pH
pH=5-6.8
pH triggered release of uPA at acidic condition
Typsin
(b)
Binding of uPA-polymer conjugates with activated platelets and locally liberated uPA
Targeted thombolysis by pH triggered release of uPA

Challenges and Limitations

- Safety and Long-Term Toxicity
- Manufacturing and Scalability Challenges
- Regulatory and Approval Barriers
- Pharmacokinetic and Biodistribution Uncertainty
- Cost and Health-Economic Considerations
- Stability and Storage Issues


Thanks for your attention