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ILLUSTRATED REVIEW

Polyphosphate in thrombosis, hemostasis, and inflammation

Catherine J. Baker MS | Stephanie A. Smith DVM | James H. Morrissey PhD

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan

Correspondence
James H. Morrissey, Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI.
Email: jhmorris@umich.edu

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Abstract

This illustrated review focuses on polyphosphate as a potent modulator of the plasma clotting cascade, with possible roles in hemostasis, thrombosis, and inflammation. Polyphosphates are highly anionic, linear polymers of inorganic phosphates that are widespread throughout biology. Infectious microorganisms accumulate polyphosphates with widely varying polymer lengths (from a few phosphates to over a thousand phosphates long), while activated human platelets secrete polyphosphate with a very narrow size distribution (about 60-100 phosphates long). Work from our lab and others has shown that long-chain polyphosphate is a potent trigger of clotting via the contact pathway, while polyphosphate of the size secreted by platelets accelerates factor V activation, blocks the anticoagulant activity of tissue factor pathway inhibitor, promotes factor XI activation by thrombin, and makes fibrin fibrils thicker and more resistant to fibrinolysis. Polyphosphate also modulates inflammation by triggering bradykinin release, inhibiting the complement system, and modulating endothelial function. Polyphosphate and nucleic acids have similar physical properties and both will trigger the contact pathway—although polyphosphate is orders of magnitude more procoagulant than either DNA or RNA. Important caveats in these studies include observations that nucleic acids and polyphosphate may co-purify, and that these preparations can be contaminated with highly procoagulant microparticles if silica-based purification methods are employed. Polyphosphate has received attention as a possible therapeutic, with some recent studies exploring the use of polyphosphate in a variety of formulations to control bleeding. Other studies are investigating treatments that block polyphosphate function as novel antithrombotics with the possibility of reduced bleeding side effects.

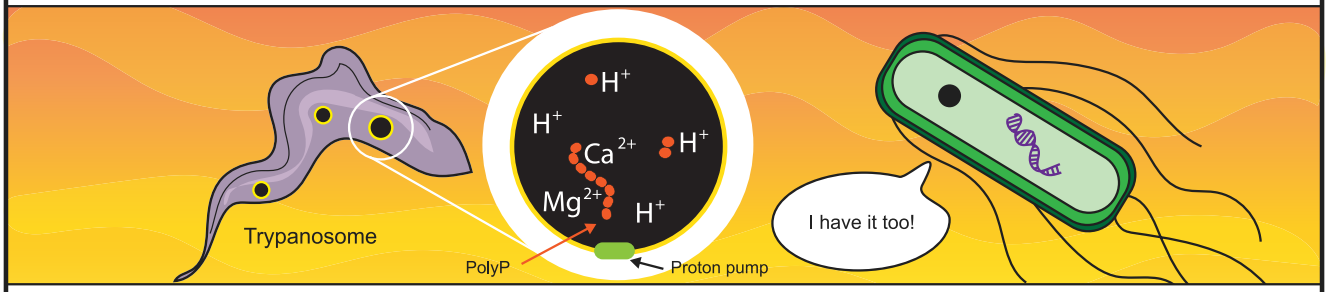
KEYWORDS
blood coagulation, contact pathway, DNA, nucleic acids, polyphosphate, RNA

Essentials

- Polyphosphate is present in microorganisms and human cells such as platelets.
- Polyphosphate modulates coagulation via interactions with multiple proteins.
- Polyphosphate modulates inflammation by triggering bradykinin release and inhibiting complement.
- Nucleic acids and polyphosphate co-purify and may be contaminated with silica-based methods.

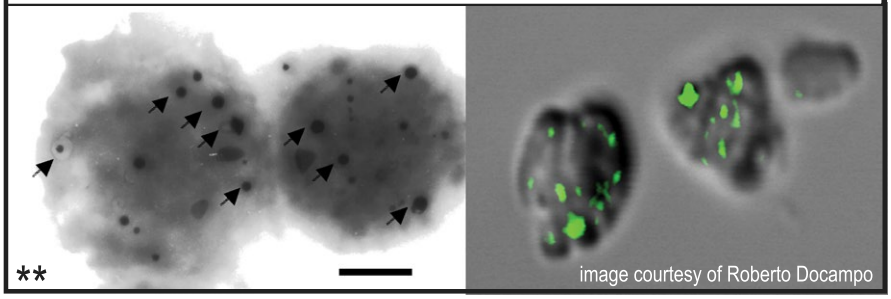
Where is polyP?

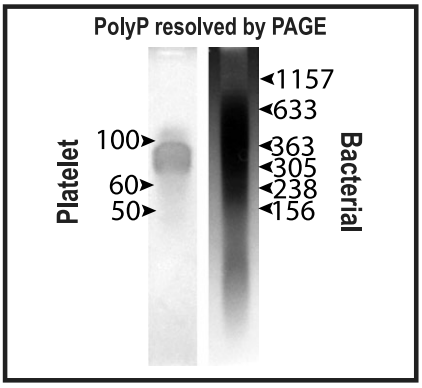
In microbes, polyP size is heterogeneous, ranging from a few phosphates to over a thousand phosphates in length.[1]



It's stored in acidocalcisomes (eukaryotes) or volutin granules (bacteria), along with divalent metals and amines.[2,3]

Platelet dense granules are similar in composition to acidocalcisomes and have abundant polyP. Its polyP length is tightly regulated (60-100 phosphates long).[4]

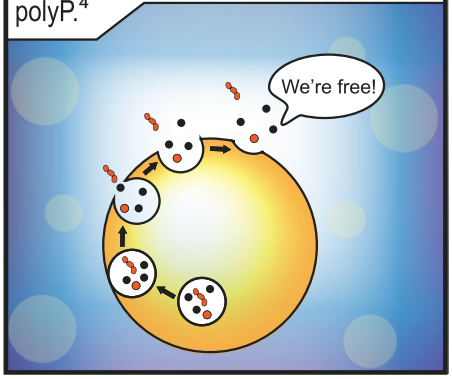


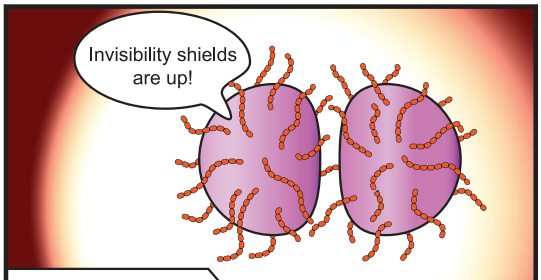


PolyP is also reported in mast cells,[5] prostasomes,[6] cardiac muscle,[7,8] brain,[9] and nervous tissue.[10]

How does it come in contact with blood?

Platelets, when activated, secrete all their granule contents— including polyP.[4]





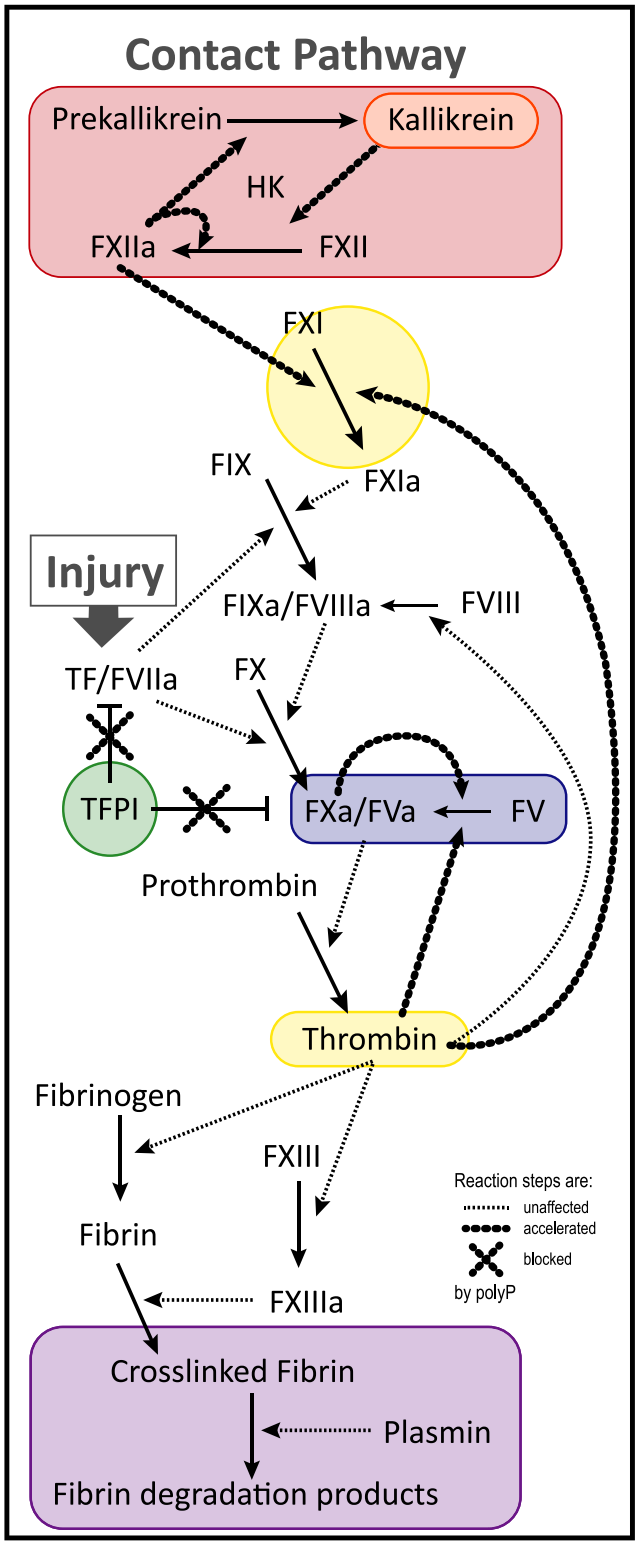
Some organisms (such as *Neisseria*, a causative agent of meningitis) express polyP on their capsule surface.[11] It is a virulence factor that may allow them to evade complement killing.[12,13]

PolyP may be released following tissue damage or cell necrosis.

PolyP is very short (25mer) in cardiac muscle,[7-8] slightly longer in platelets[4] and mast cells[5] (60-100mer). Some tissues, such as brain,contain very long polymers (800mer).[9]

PolyP is widespread in infectious microorganisms and is released by activated platelets and mast cells. It may also be released following tissue damage. In these settings, it may act as a pathogen-associated molecular pattern (PAMP) or a damage-associated molecular pattern (DAMP) to help trigger host defenses.

Roles of polyP in coagulation & kinin generation



PolyP is a contact activator

Long-chain (microbial) polyP triggers clotting by providing a template for autoactivation and reciprocal activation of the proteins in the contact pathway.[14,15] The enzymes, FXIIa and kallikrein, are generated. Platelet-size polyP supports this reaction poorly.[14]

PolyP causes bradykinin release

Newly generated kallikrein cleaves high molecular weight kininogen to release bradykinin, which is a potent vasodilator and proinflammatory mediator.[16]

PolyP greatly accelerates FXI activation

FXI deficiency is associated with bleeding, indicating that FXI activation plays a role in hemostasis.[17] While thrombin activation of FXI is slow,[18,19] platelet-size polyP enhances its rate 3000-fold, making this back-activation reaction a physiologically relevant contributor to sustained thrombin generation.[20,21]

PolyP blocks TFPI activity

Tissue Factor Pathway Inhibitor (TFPI) antagonizes tissue factor-dependent initiation of coagulation by inhibiting FXa and FVIIa. Platelet-size polyP potently abrogates TFPI's anticoagulant function[14,22] and enhances the inactivation of TFPI by FXIa.[23]

PolyP accelerates thrombin generation

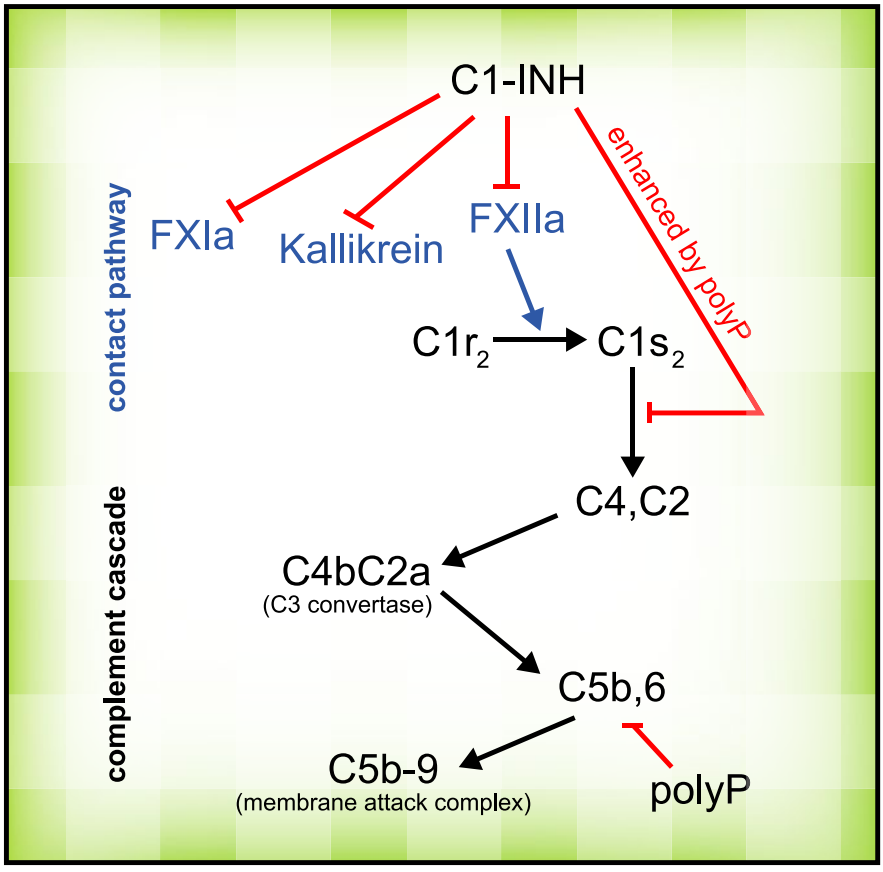
FV activation is a rate-limiting step in thrombin generation. Because platelet-size polyP enhances the rate of FV cleavage by FXIa, FXa and thrombin, the kinetics of thrombin generation are improved.[22,24]

PolyP strengthens clots and delays lysis

PolyP is incorporated into fibrin, leading to thicker fibrin fibrils that are more resistant to fibrinolysis.[14,25,26]

PolyP accelerates blood clotting by targeting a few specific points in the clotting cascade, always in a procoagulant manner. The exact steps modulated by polyP depend on its polymer length.

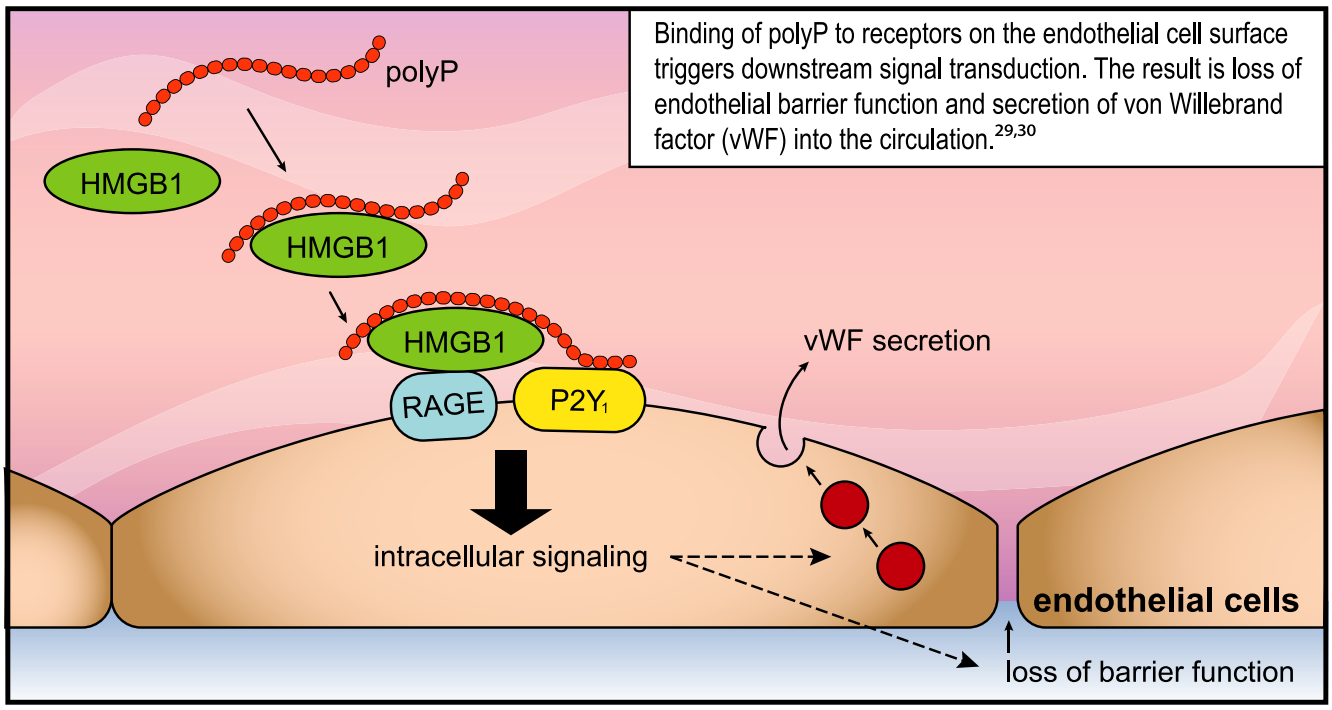
Roles of polyP outside of coagulation



Although C1 esterase inhibitor (C1-INH) is a promiscuous serpin which can inhibit members of both the contact pathway (FXIa, FXIIa, and kallikrein) and the complement cascade (C1s), polyP only enhances the inhibitory effect of C1-INH toward complement,[27] not toward clotting factors.

PolyP also destabilizes the C5b,6 complex, thereby reducing the lytic capacity of the membrane attack complex of the complement system.[28]

The overall effect of polyP is down-regulation of the complement system, which is opposite to the effect it has on the clotting cascade.[28]

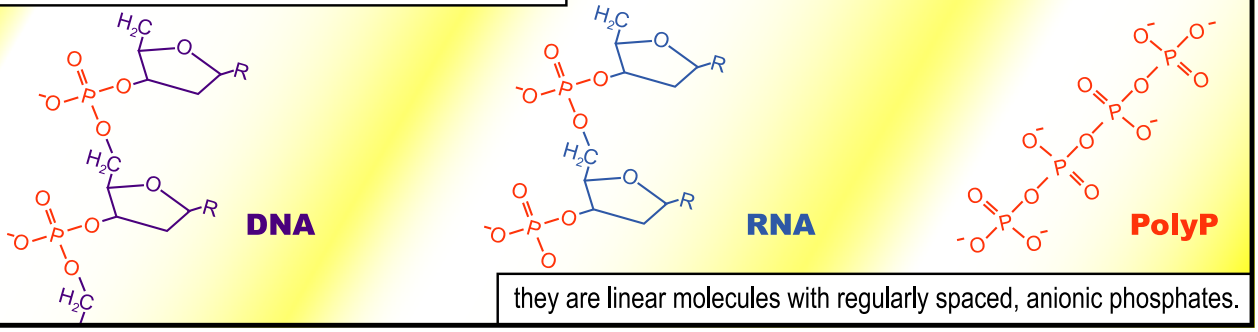


Binding of polyP to receptors on the endothelial cell surface triggers downstream signal transduction. The result is loss of endothelial barrier function and secretion of von Willebrand factor (vWF) into the circulation.[29,30]

It's likely that additional roles of polyP in inflammation and vascular function are yet to be found.

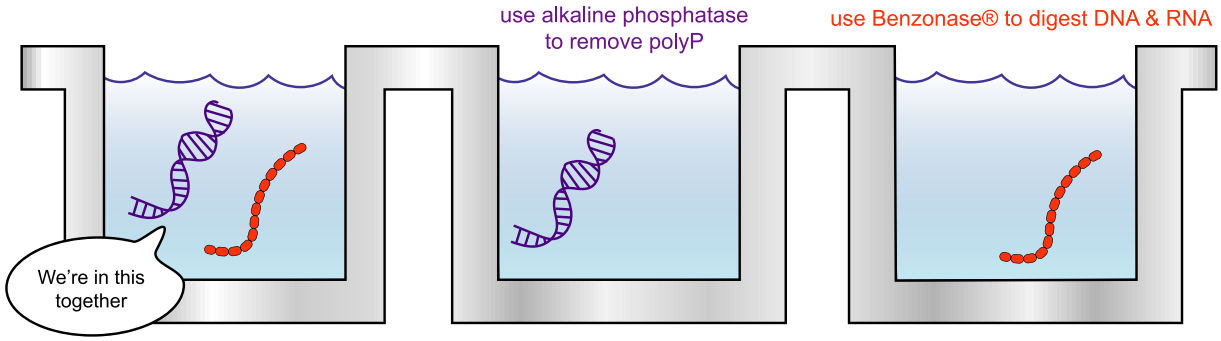
Comparison between DNA, RNA, and polyP

Nucleic acids and polyP have similar physical properties

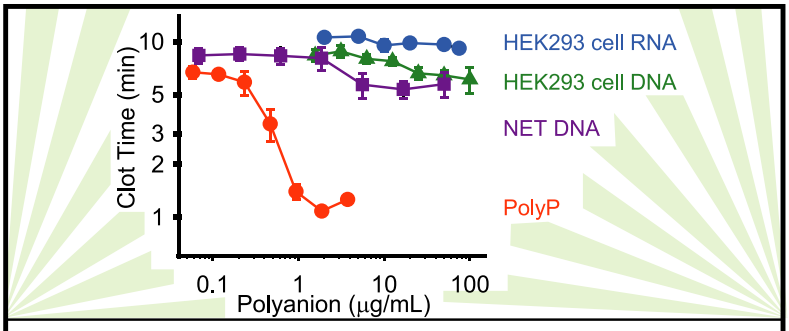


they are linear molecules with regularly spaced, anionic phosphates.

Due to these similarities, polyP will often co-purify with nucleic acids.[31]

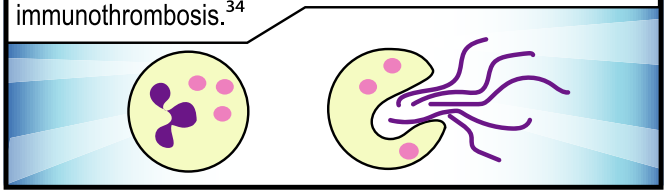


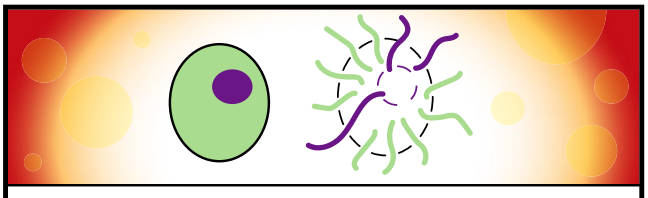
Also, some widely used methods to purify these polymers can shed silica particles.[32]



But when you remove those confounders, polyP is orders of magnitude more active than DNA or RNA at activating the contact system.[31]

DNA released from immune cells is part of the inflammatory response to infection.[33] NETosis is a component of immunothrombosis.[34]



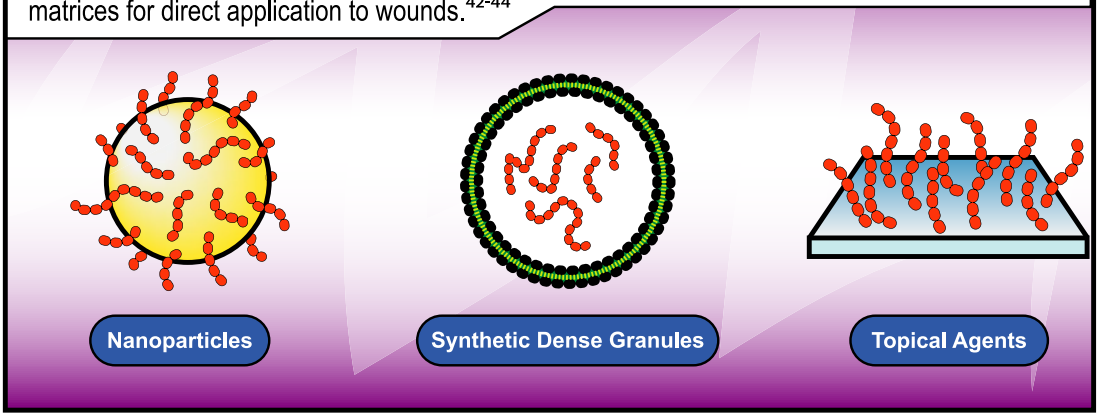


Necrosis also releases nucleic acids and polyP, bringing them into contact with clotting proteins in plasma.[35]

Extracellular polyP, DNA, and RNA can be procoagulant and proinflammatory, but their contribution to these processes requires much further investigation!

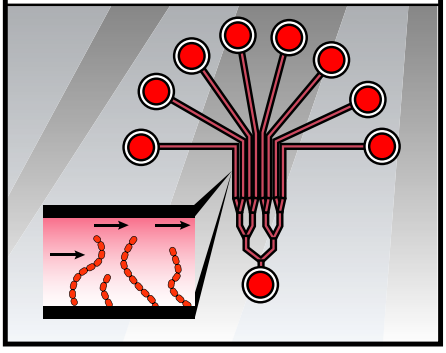
PolyP as a possible therapeutic or target

To treat bleeding? PolyP has been covalently attached to particles or surfaces[36-40] and encapsulated in liposomes[41] for targeted delivery at the site of injury. It has also been attached to matrices for direct application to wounds.[42-44]



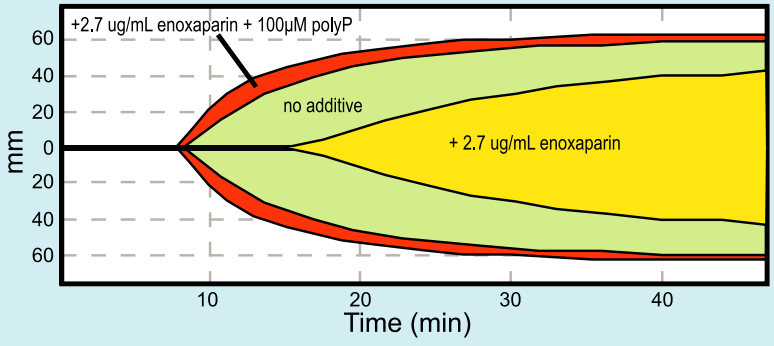
Because polyP enhances fibrin clot structure,[25] it could be used to augment treatment of surgical bleeding with fibrin glue.

Ex vivo investigations of whole blood using microfluidics are helping define polyP's contributions to hemostasis and thrombosis under more realistic conditions.[37,45] These studies also test potential polyP inhibitors.[45-46]

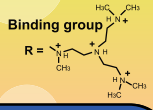


Adding polyP to blood in vitro reverses the anticoagulant effects of heparins and DOACS (direct FXa or thrombin inhibitors). PolyP also mitigates the anti-coagulant effects of vitamin K antagonists and enhances clotting in hemophilic plasma.[47]

Whole blood thromboelastometry showing the reversal of enoxaparin by polyP

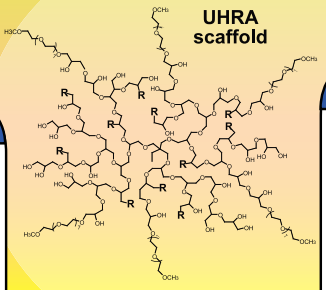


To treat or prevent thrombosis? Various compounds antagonize the procoagulant effects of polyP, both in vitro and in vivo. Molecules that bind polyP via ionic interactions prevent its modulation of coagulation.[46,48] Alternatively, polyP can be enzymatically digested.



Proteins

- scPPX, a yeast-derived exopolyphosphatase[49]
- Alkaline phosphatases[50]
- PPXbd, the polyP-binding domain from *E. coli* exopolyphosphatase[45,48,51]



Cationic Polymers

- Polyethyleneimine[48]
- Polymyxin B[48]
- PAMAM dendrimers[48,52]
- Universal Heparin Reversal Agents (UHRAs)[46]

Some researchers are incorporating polyP into novel hemostatic agents to control bleeding. Also, because polyP enhances, but is not essential for, coagulation, it represents an attractive target for thrombosis prevention and treatment.

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RELATIONSHIP DISCLOSURES

SAS and JHM hold patents related to the potential medical uses of polyphosphate and polyphosphate inhibitors. JHM has equity ownership in PrevThro Pharmaceuticals and consults for Cayuga Pharmaceuticals.

AUTHOR CONTRIBUTIONS

CJB created the graphics; SAS contributed images and wrote the text; CJB, SAS, and JHM contributed to the conceptual design and edited the manuscript.

ORCID

Catherine J. Baker http://orcid.org/0000-0001-5299-1465
Stephanie A. Smith http://orcid.org/0000-0003-4895-6215
James H. Morrissey http://orcid.org/0000-0002-1570-1569

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