CENTER FOR BIOENGINEERING
UC SANTA BARBARA

adapted from
CBE/ENGR 225 FACULTY SEMINAR
February 9, 2016

Learning about the blood coagulation system from the outside and inside with simple, low-cost inorganic materials

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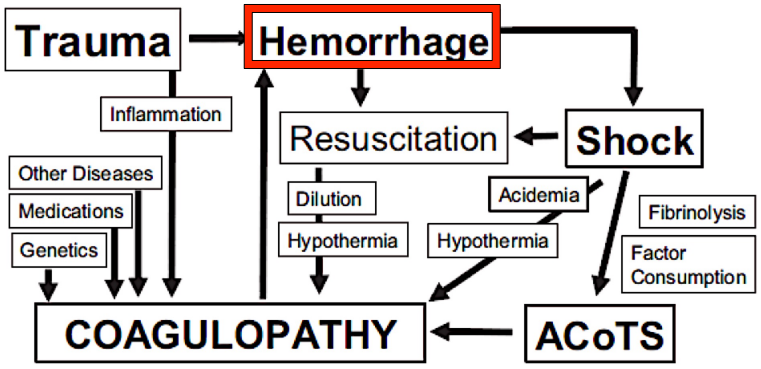
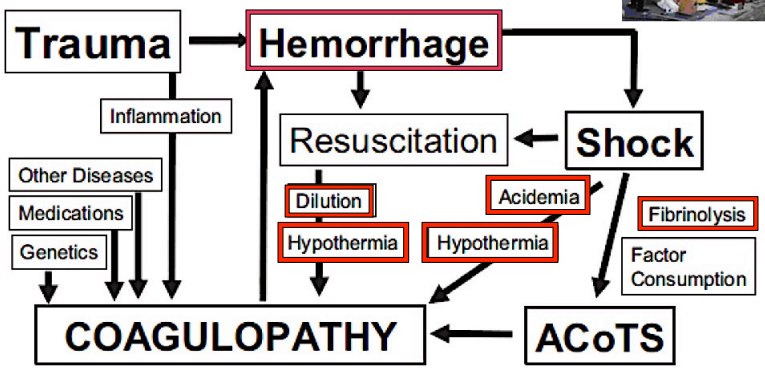
Open Wound Arterial Bleeding –Penetrating Trauma

Average adult male human has approximately 5-6 L of blood
Heart can pump 4-5 L blood /minute
Mortality after 1.6-2 L blood loss
QUICK INTERVENTION PREVENTS DEATH 3-5 minutes or less



J. R. Hess et al., The journal of TRAUMA Injury, Infection, and Critical Care, 2008
D. Frith, M. J. Cohen, K. Brohi, Thrombosis Research 129 (2012) 551

Blunt Trauma Trajectory -- internal bleeding & long time line



Coagulopathy : Blood's ability to clot is impaired

Genetic: How well does your body regulate clotting-anticlotting?

J. R. Hess et al., The journal of TRAUMA Injury, Infection, and Critical Care, 2008

D. Frith, M. J. Cohen, K. Brohi, Thrombosis Research 129 (2012) 551-556

The Challenge

♦ **Open wound, rapid response treatment -- short time window**

Stop arterial hemorrhaging in < 5 minutes by interfacing the blood clotting system with an externally applied agent

♦ **Internal bleeding -- trauma trajectory --- longer time window**

Selective, targeted delivery into blood coagulation system -- therapeutic

♦ **Traumatic Coagulopathy -- abnormal propensity toward bleeding**

Lethal Triad ⟷ Hypothermia – Acidosis – Dilution

History

As of 2006, still no solution to deaths from open wound arterial bleeding

"Uncontrolled hemorrhage continues to be the leading cause of death due to military trauma and the second leading cause of death in the civilian setting"

Pusateri, Holcomb, Kheirabadi, Alam, Wade, and Ryan, Journal of TRAUMA *Injury, Infection, and Critical Care,* 2006; *60*, 674

The Starting Point – State of the Art
Open Wound Arterial Bleeding 2004-2006

Dehydrated zeolite hemostatic agent achieves 100% survival in a lethal model of complex groin injury in swine

Alam, Burris, DaCorta,Rhee *Military Medicine* 2005, 170, 63
Ostomel, Stoimenov, Holden, Alam, Stucky *J Thrombosis & Thombolysis* 2006, 22, 55

Sent to Iraq for Military Medical Command use

Applied as a particulate powder, QuikClot

Z-Medica Wallingford, CT

Why zeolite for hemostasis?

Emma Maris, *Nature* (2006) 446, 369

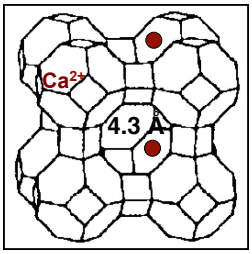
"QuikClot powder is made of porous minerals called zeolites. The story is that inventor Frank Hursey, who was working with zeolites as sieves to separate gases (N_2 from O_2), cut himself shaving and applied it to his face on a whim. How it works is still unclear, although it has been approved for clinical use. "There is a whole lot of surface chemistry," says Huey (CEO Z-Medica). The product also includes calcium ions, catalysts for the body's clotting process."

Zeolite Ca 5A

$NaCa_{5.5}(SiO_2)_{12}(AlO_2)_{12}$

• 27 H_2O (when fully hydrated)

Surface Area = 571 m^2/gm

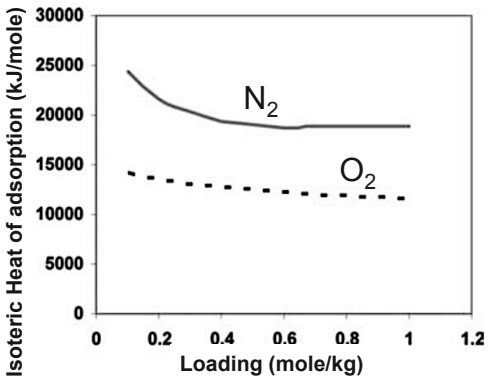


F. X. Hursey, F.J. Dechene, Method of Treating Wounds; Patent 4,822,349 (1989)

Air Products

Commercial separation of N_2 from O_2

Using Zeolite 5A confined space electrostatic field



4:1 selectivity

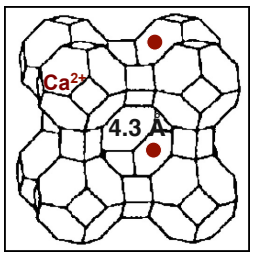
J. Chem. Eng. Data 2009, 54, 916

Electric Field

3.22 V/Å @ 2.5 Å

5.65 V/Å @ 2.0 Å

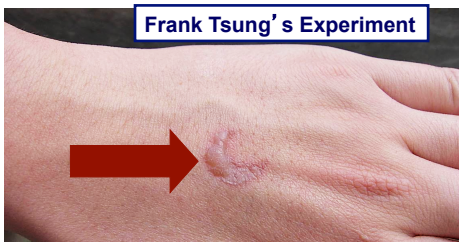
Zeolite Ca 5A



A Problem

$\Delta H_{hydration}$ = 680 J/g !

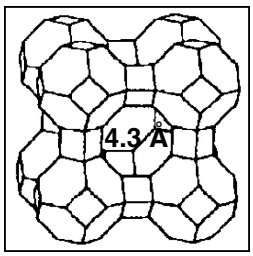
2nd and 3rd degree burns on application



How does it stop bleeding?

Cauterization?
Ca^{2+} delivery?

Zeolite Ca 5A



$NaCa_{5.5}(SiO_2)_{12}(AlO_2)_{12} \cdot 27H_2O$
(when fully hydrated)

Hemostasis without burns?

April Sawvel

Sarah Baker

Todd Ostomel

Rapid Response – Open Wound

University -- Medical Research Hospitals --Industry Collaboration

April Sawvel
Todd Ostomel
Sarah Baker
UCSB

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Harvard Medical School

Dr. Richard McCarron
Naval Medical Research Center

Bijan S. Kheirabadi, PhD
Michael R. Scherer, MA
J. Scot Estep, DVM,
Michael A. Dubick, PhD,
John B. Holcomb, MD

Institute of Surgical Research
Fort Sam Houston

Giacomo Basadonna, M.D.
University of Massachusetts
Medical School

Raymond J. Huey
Denny Lo

Z-MEDICA

Interface Bioprocess Control of Blood Clotting Cascade

2004

Stucky Research Group
Material Synthesis
Interface Control Design
***In-Vitro* Blood Testing**

Naval Medical Research Center
Secondary *In-Vitro* Testing
***In-Vitro* Protocol Validation**
Swine Model *In-Vivo* Testing

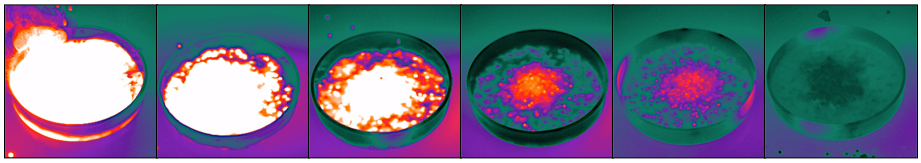
Z-Medica
Product Development
Packaging
***In-Vivo* Testing -FDA**

Field Use

Institute of Surgical Research
Fort Sam Houston

Starting in 2007

Two effective ways to tune heat release

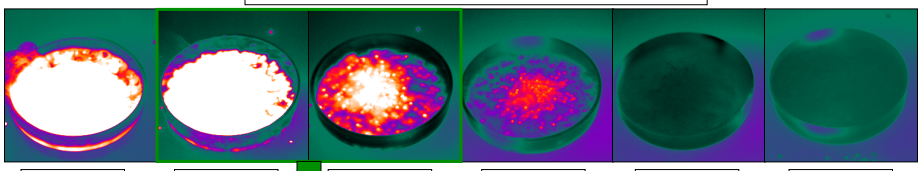


96.5 °C | 75.7 °C | 37.2 °C | 24.5 °C | 23.3 °C | 19.6 °C

Increasing Pre-Hydration

0% | 10% | 20% | 40% | 80% | 100%

0.37% Ag-Exchanged Zeolite 5A

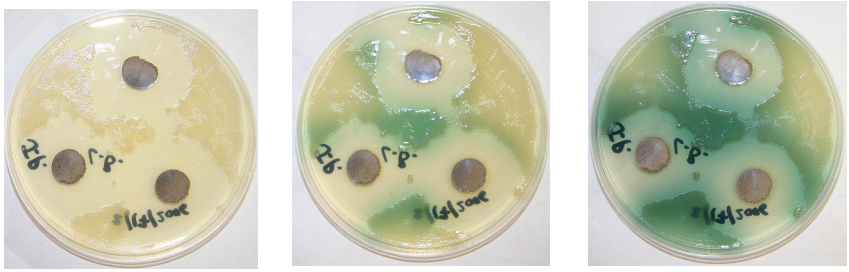


96.9 °C | 68.4 °C | 29.2 °C | 24.0 °C | 19.5 °C | 19.2 °C

Optimal Pre-Hydration Range

Antibiotic Activity

F1:$Ag_{1.308}Na_{0.230}Ca_{5.230}(SiO_2)_{12}(AlO_2)_{12}\bullet xH_2O$

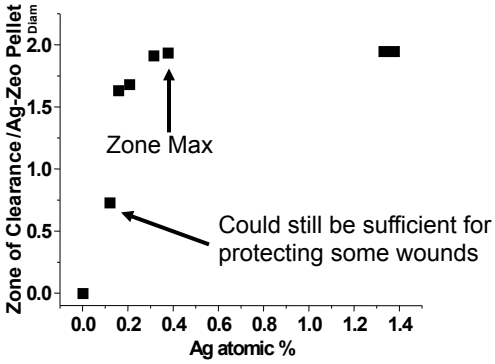


24 hours | 48 hours | 72 hours

Zone of No Growth Surface Area : Pellet Geometric Surface Area after 24 hours ~ 2.2 cm^2 for Ag-exchanged LTA-5A

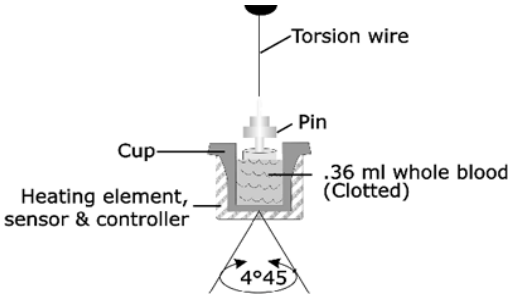
Antibiotic Activity as a function of Ag content

Ag ion delivery



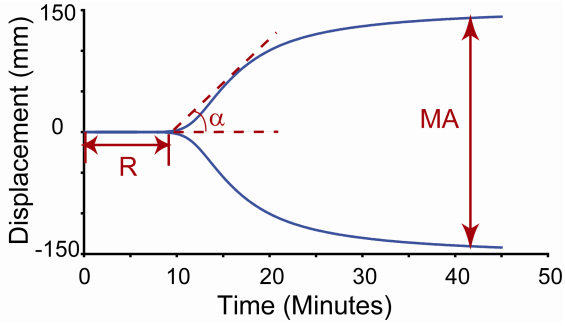
TA Ostomel, PK Stoimenov, PA Holden, HB Alam, GD Stucky. *J. Throm Thrombolys*, 2006, 22, 55

In Vitro Testing UCSB

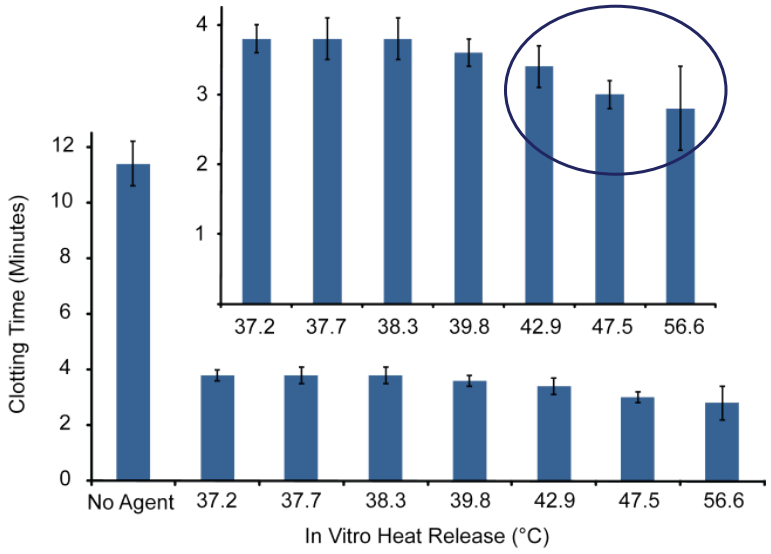


Haemoscope®
Thrombelastograph

R = Clotting Time
α = Rate of Clotting
MA = Clot Strength

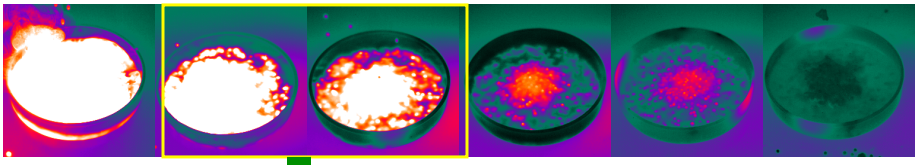


Clotting times in whole blood decrease with increasing temperature



Thermal Imaging and Heat Release

$NaCa_{5.5}(SiO_2)_{12}(AlO_2)_{12}•xH_2O$

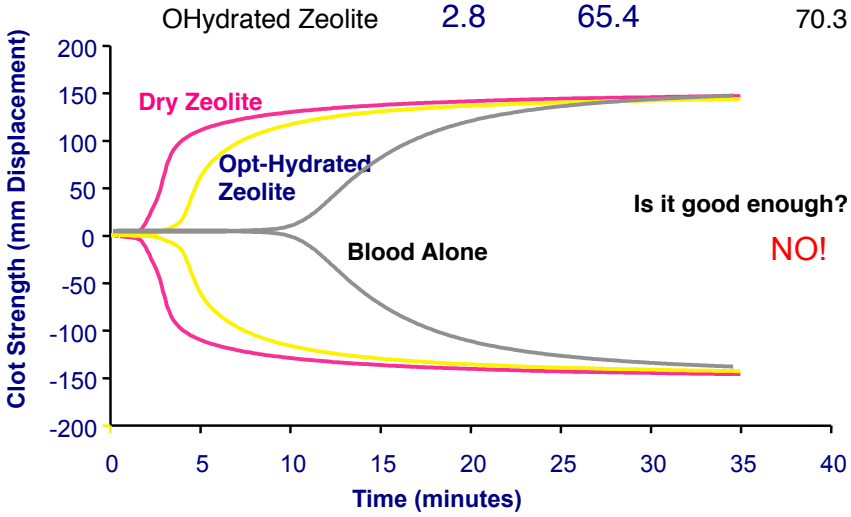


96.5 °C | 75.7 °C | 37.2 °C | 24.5 °C | 23.3 °C | 19.6 °C

Optimal Pre-Hydration Range
3 to 5 wt %
42 to 45 ˚C

Dehydration effect

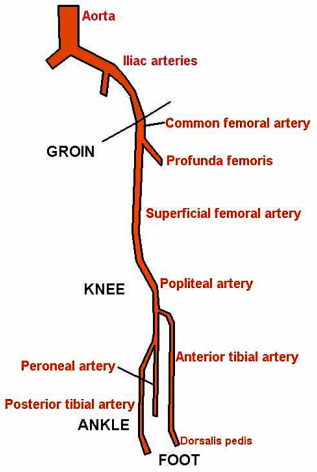
Sample	R (minutes)	α (degrees)	MA (mm) Displacement
Blood Alone	10	53.4	72.7
Dry Zeolite	1.4	74.0	71.4
OHydrated Zeolite	2.8	65.4	70.3



In Vivo Testing

Uniformed Services University Hospital, Bethesda, MD

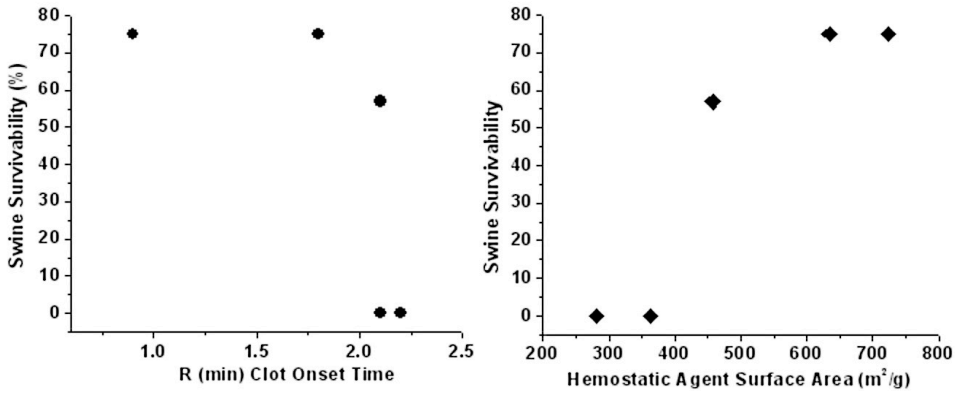
In Vivo Simulated Traumatic Swine Injury Testing



N Ahuja, TA Ostomel, HB Alam, GD Stucky, *J Trauma*, 2006

In Vitro Parameters and In Vivo Performance

Selection Criteria for Hemostatic Agents



Field conclusion --- Not good enough!

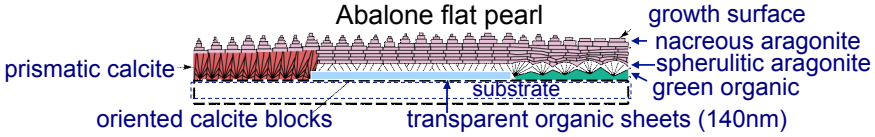
Need better *in vivo and field survivability*

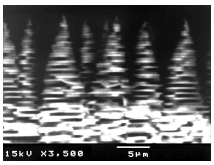
TA Ostomel, PK Stoimenov, P Holden, HB Alam, GD Stucky. Host-Guest Composites for Induced Hemostasis and Therapeutic Healing in Traumatic Injuries. *J Thromb Thrombolys.* 2005

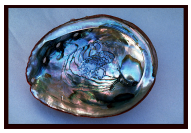
What Next?

How might the inorganic agent "control" the blood clotting cascade biosystem?

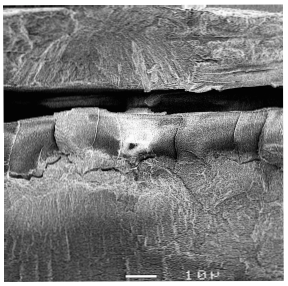
Inorganic Control of a BioProcess—Abalone shell growth



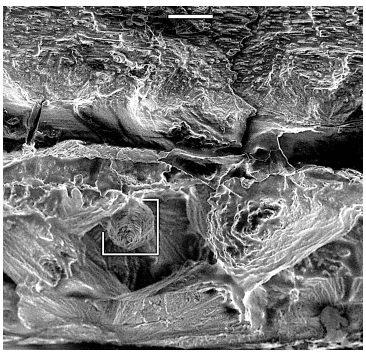




large $\Delta G_{crystal}$



Growth on calcite or glass substrate



Growth on MoS_2

Zaremba, Belcher, Chem. Mater. 8, 679 (1996)

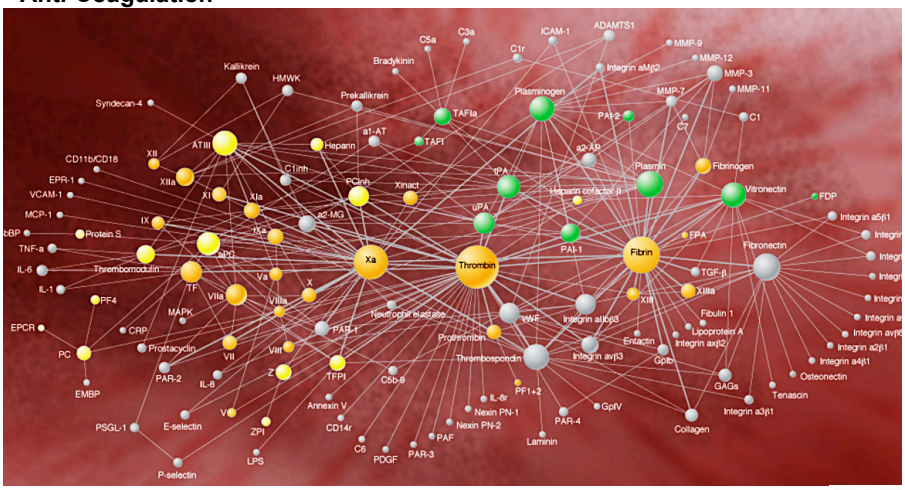
Interface with Blood Clotting Cascade System?

- Coagulation
- Fibrin Formation
- Anti-Coagulation

> 220 factors
> 300 interactions

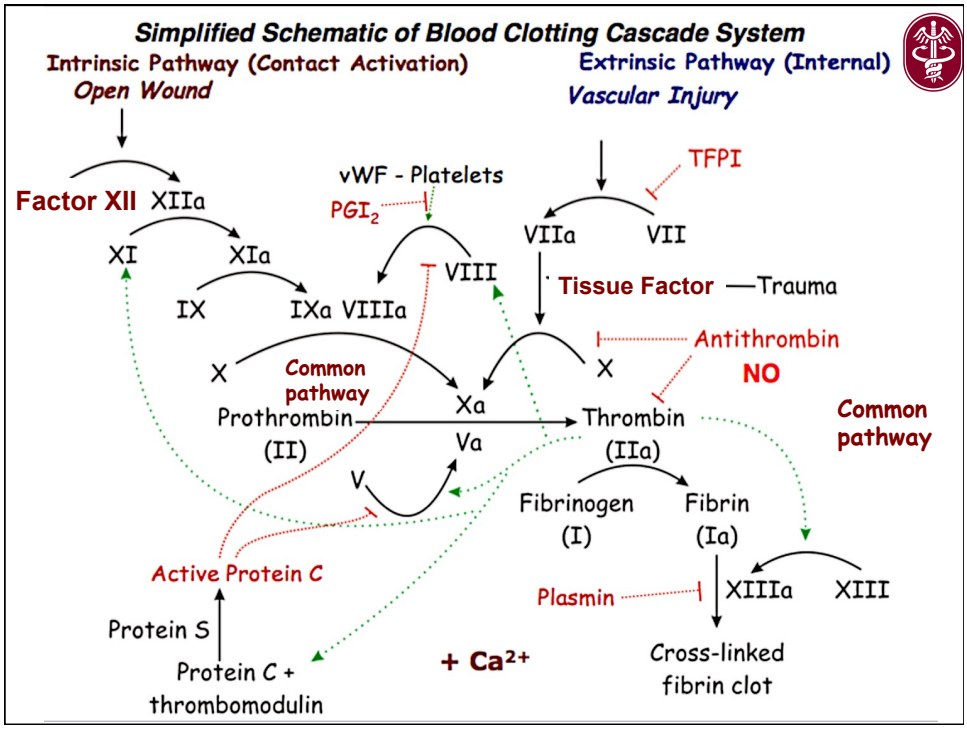
+

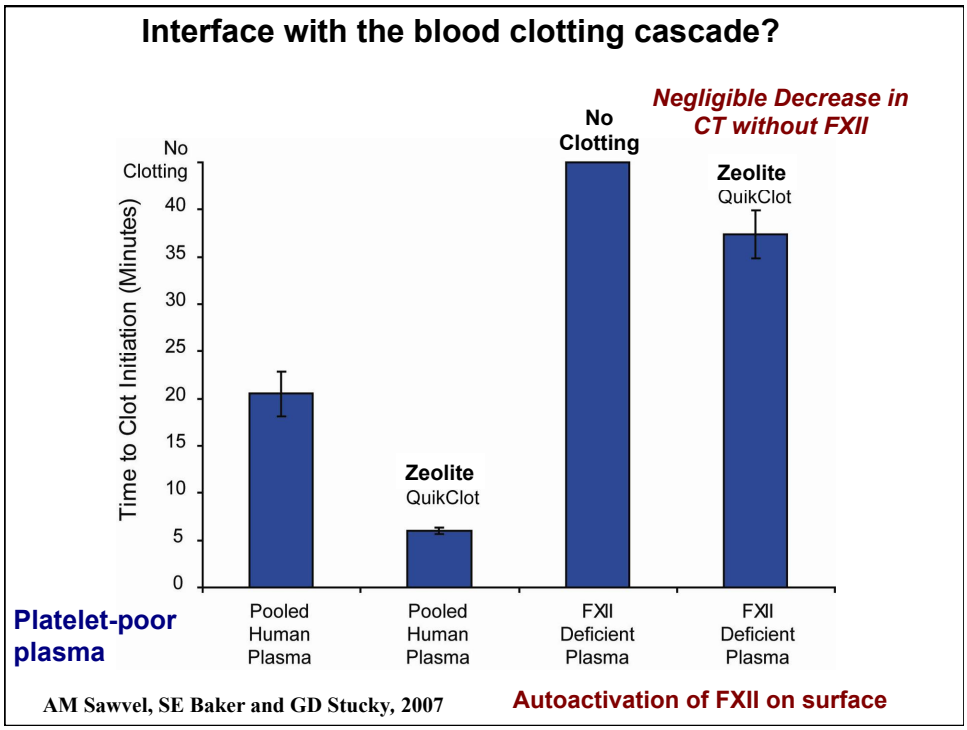
- Warfarin, heparin
- Dilutional coagulopthy
- Acidosis pH < 7.35
- Hypothermia



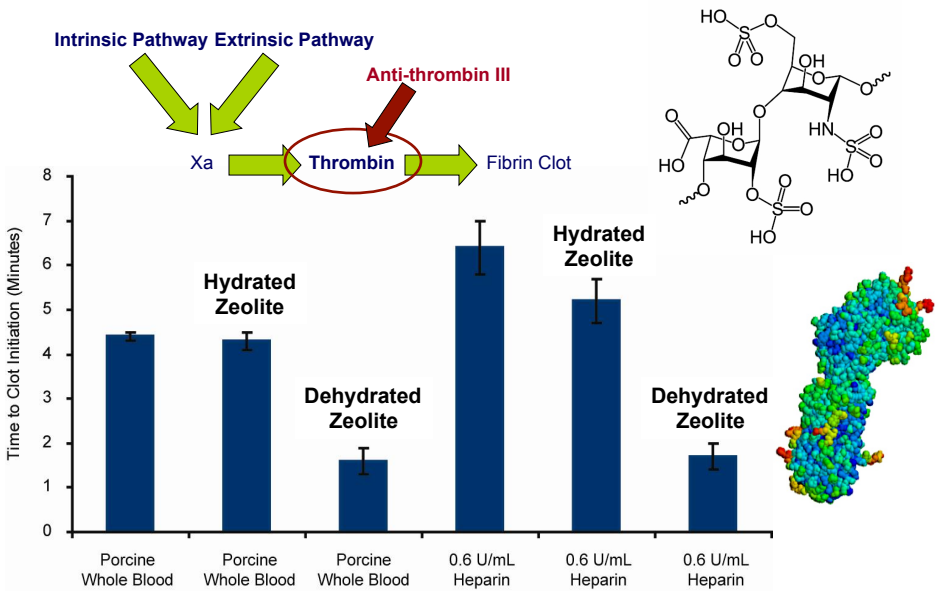
Jeff Varner **Cornell**
Mitchell Cohen UCSF
Mol. BioSyst. 2010, 6, 2272

Frank Doyle UCSB
Linda Petzold UCSB


Simplified Schematic of Blood Clotting Cascade System
Intrinsic Pathway (Contact Activation)
Open Wound
Extrinsic Pathway (Internal)
Vascular Injury
Factor XII
XIIa
XI
XIa
IX
IXa
VIIIa
vWF - Platelets
PGI2
VIII
TFPI
VIIa
VII
Tissue Factor
Trauma
Antithrombin
NO
X
Common pathway
Prothrombin (II)
Xa
Va
Thrombin (IIa)
Common pathway
V
Fibrinogen (I)
Fibrin (Ia)
XIIIa
XIII
Active Protein C
Plasmin
Protein S
Protein C + thrombomodulin
+ Ca2+
Cross-linked fibrin clot


Interface with the blood clotting cascade?
Negligible Decrease in CT without FXII
No Clotting
Zeolite QuikClot
Time to Clot Initiation (Minutes)
0
5
10
15
20
25
30
35
40
Zeolite QuikClot
Platelet-poor plasma
Pooled Human Plasma
Pooled Human Plasma
FXII Deficient Plasma
FXII Deficient Plasma
AM Sawvel, SE Baker and GD Stucky, 2007
Autoactivation of FXII on surface

Clotting in the Presence of Heparin



http://en.wikipedia.org/wiki/Antithrombin_III
SE Baker, AM Sawvel, and GD Stucky.

http://en.wikipedia.org/wiki/Heparin
http://www.medscape.com/viewarticle/456874_2

Therapeutic Inorganic System Components

High Surface Area Material Systems

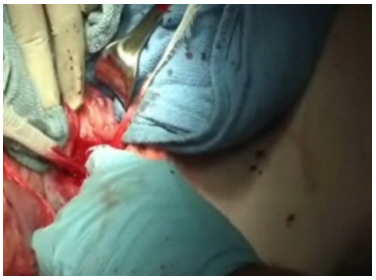
- ✓ Heat Release
- ✓ Electrolyte Transport
- ✓ Pore Structure
- ✓ Surface Area
- ✓ Surface Charge in Media
- ✓ Acidity - Basicity
- ✓ Surface Functionality
- ✓ Band Structure
- ✓ Particle Size
- ✓ Particle Morphology
- ✓ Protein Delivery/Release
- ✓ Antibiotic Delivery
- ✓ Enzyme Support Activity
- ✓ Cytotoxicity

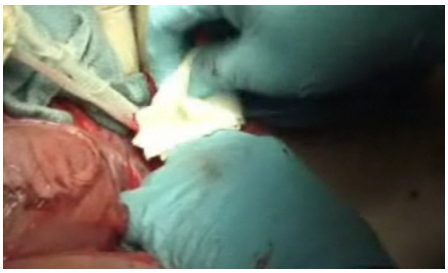
Langmuir (2008) 24, 14254
Langmuir (2007) 23, 11233
Chem Mater (2007) *19*, 4390

J Am Chem Soc (2006) 128, 8384
J. Throm Thrombolys (2006) *22*, 55
Chem Mater (2000) *12*, 686

Target: Define Response of Expanded Total System

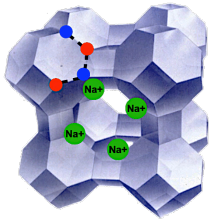
Blood clotting cascade system + **Therapeutic? inorganic System**





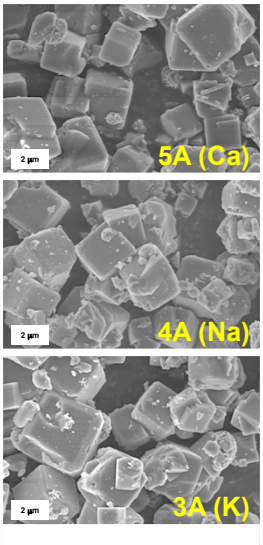
Researchers (Naval Medical Research Center) test inorganic coated gauze on an anesthetized pig

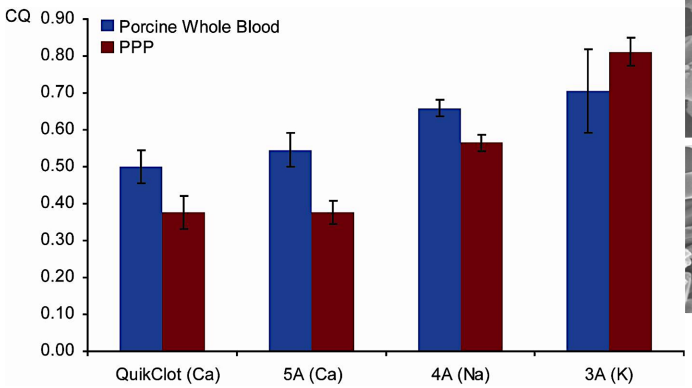
Effect of Varying the Guest Cation on Clotting Response



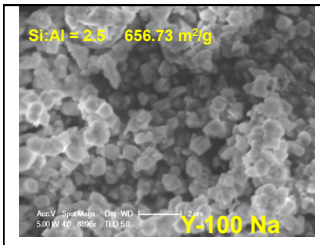
$Na^+ \longrightarrow \frac{1}{2} Ca^{2+}$

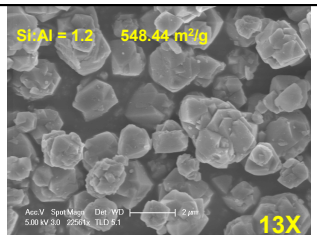
$K^+ \longrightarrow \frac{1}{2} Ca^{2+}$

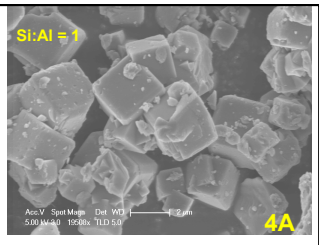


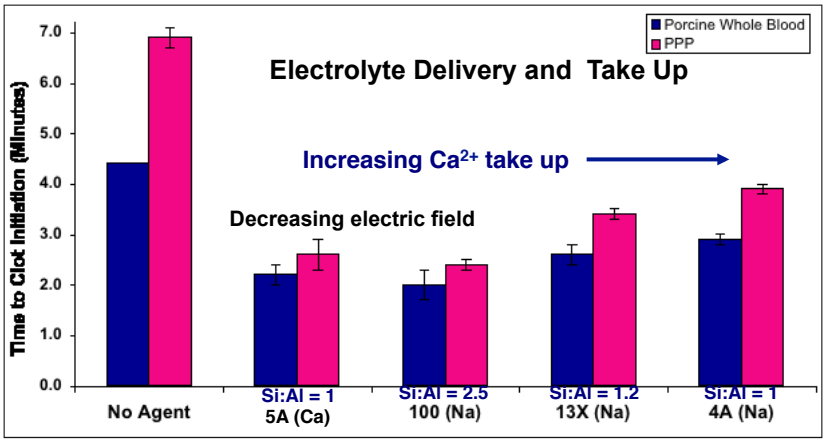


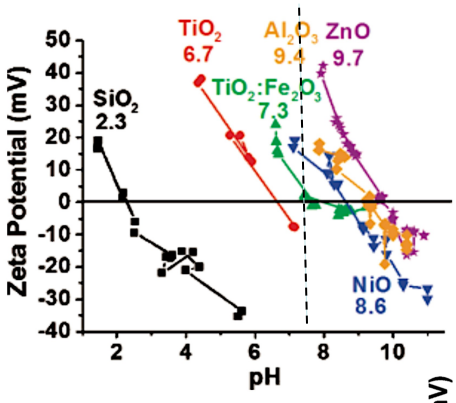
AM Sawvel, SE Baker, and GD Stucky







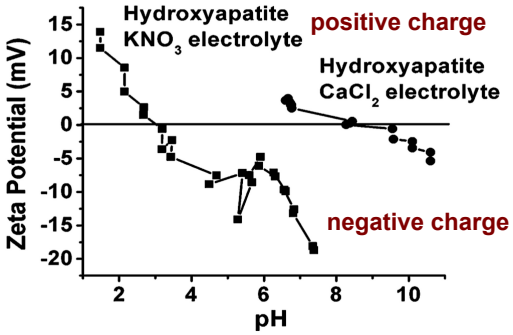




Zeta Potentials, pH and Ca^{2+}

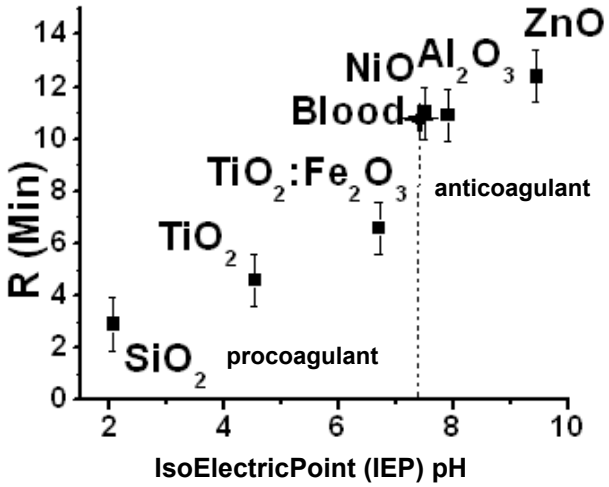
pH in $CaCl_2$(aq) electrolyte

negative charge



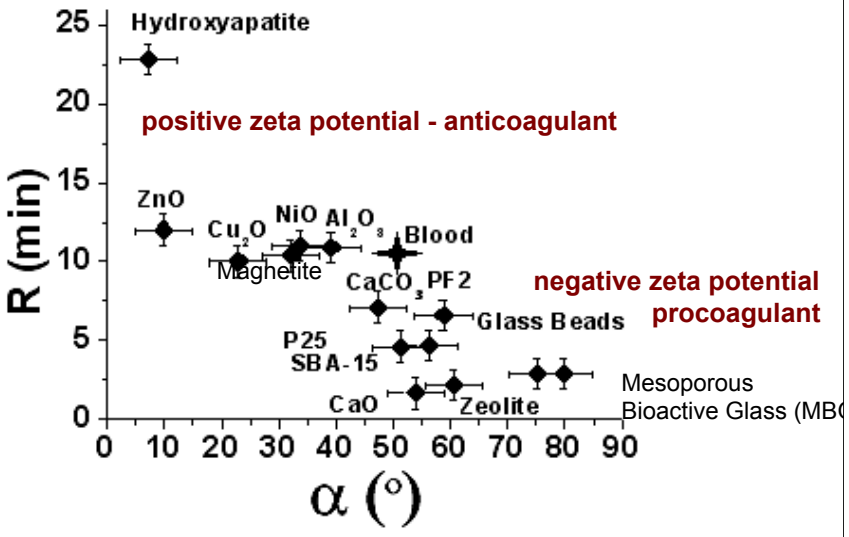
Todd A. Ostomel, Qihui Shi, Peter K. Stoimenov, and Galen D. Stucky
Langmuir **2007,** *23,* 11233

Time to Initiate Coagulation and Isoelectric Point (IEP)



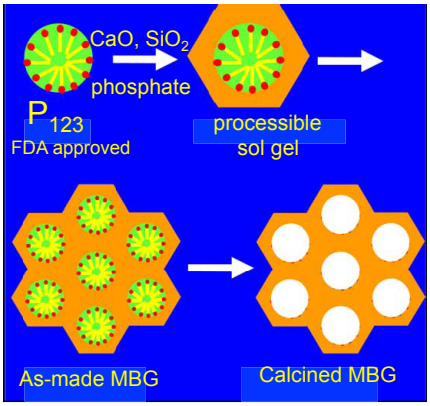
TA Ostomel, Q Shi, PK Stoimenov, GD Stucky. *Langmuir*, **2007**
TA Ostomel, Q Shi, GD Stucky. *J. Am. Chem. Soc.*, **2006,** *128*, 8384
TA Ostomel, PK Stoimenov, PA Holden, HB Alam, GD Stucky. *J. Throm Thrombolys*, **2006,** *22*, 55

In Vitro Activity with Different Oxides



Ostomel et al. 2006

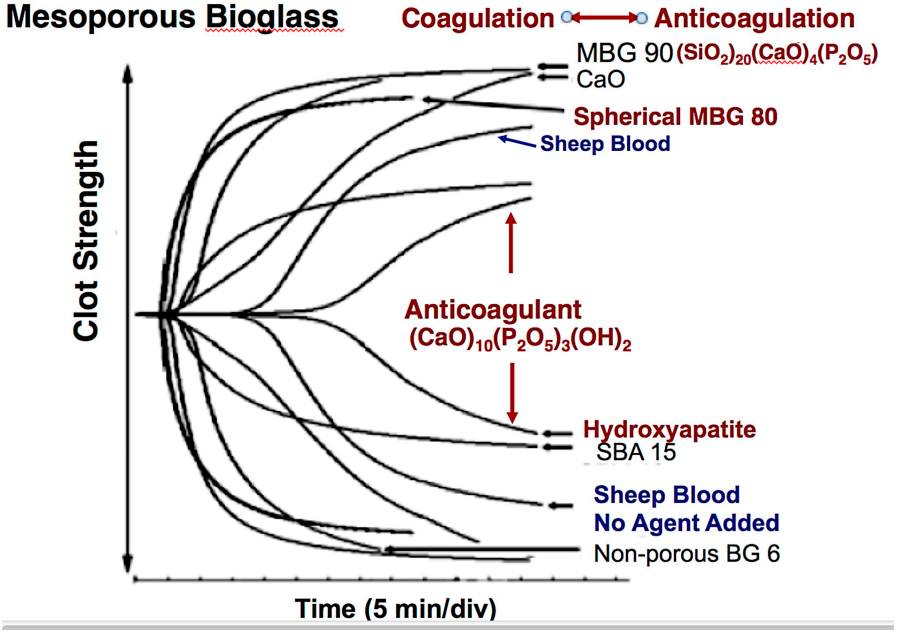
MBG: Mesoporous Bioactive Glass



- Homogeneous Composition
- High Surface Area

Ostomel, Shi, Stucky "Oxide Hemostatic Activity"*J. Am. Chem. Soc.* 2006, 128, 8384
Yan, Yu, et al. *Angew. Chem.* 2004, 43, 5980; J. Non-Crystalline Solids, 2005, 3209

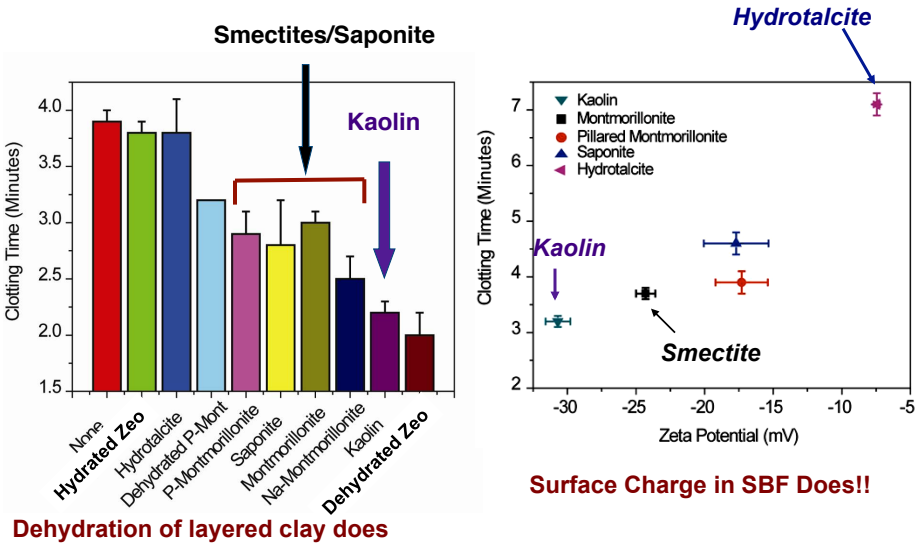
Mesoporous Bioglass



Target Parameters for First Responder Field Use

- Rapid response < 5 minutes major arterial bleeding
- Thermal Optimization of Hemostasis
- High Surface Area Efficacy
  - Light Weight
  - Antibiotic Delivery Agent
  - Therapeutic Delivery Agent - wound healing
  - Presentation of surface to support thrombosis
- Electrolyte Control (e.g., Ca^{2+}), pH Control
- Active in Presence of Heparin/Coumadin
- Biocompatible (FDA)
- **High Volume, Low Cost, Available, Simple**

Layered clays as heat free hemostatic agents? Yes and No ….

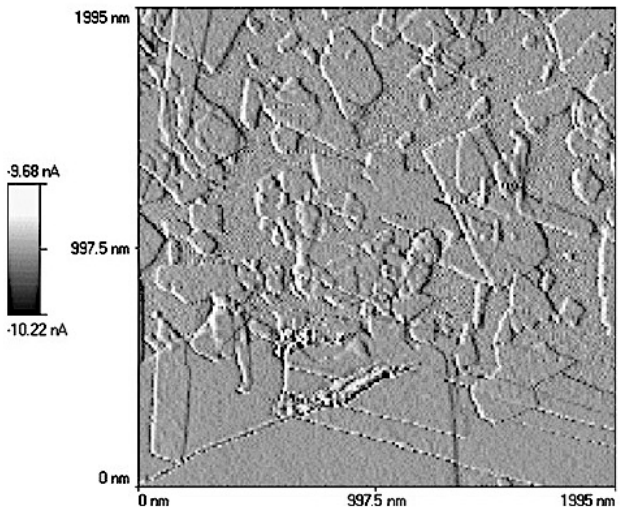


Dehydration of layered clay does not improve effectiveness

Surface Charge in SBF Does!!

Baker, Sawvel, Zheng, Stucky. *Chem. Mater.*, 2007, *19*, 4390

Smectites & Saponites –Swellable Clays very Cytotoxic



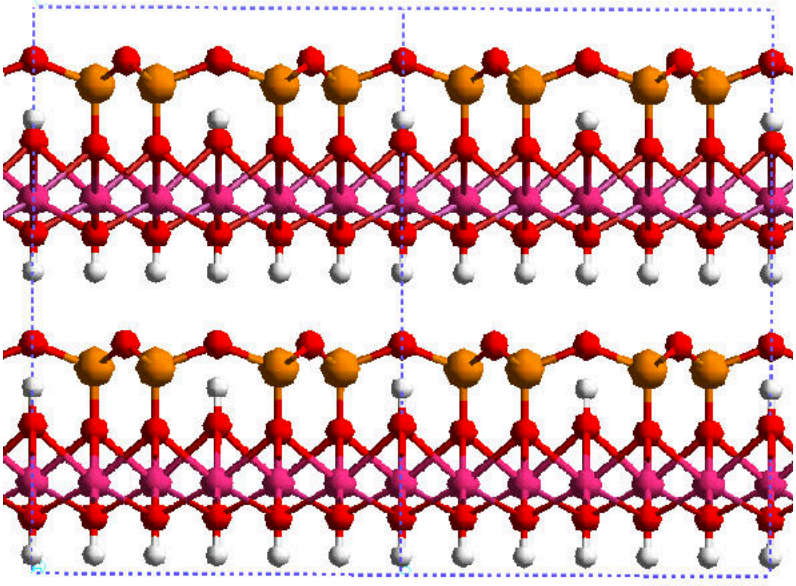
Why?

Exfoliation !

Extensive animal Testing at San Antonio Army Medical Command Research Center

R. A. Schoonheydt and co-workers, PCCP 9, 918 (2007)

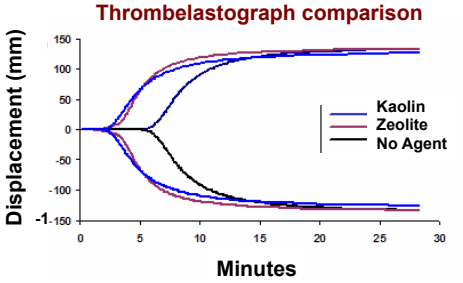
Kaolinite - $Al_2Si_2O_5(OH)_4$ Non-swellable – high charge



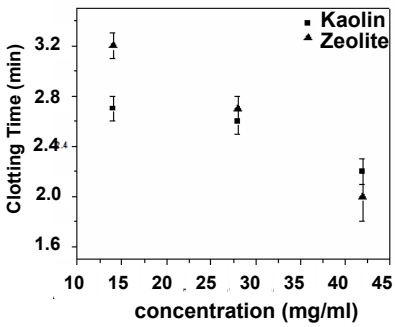
DL Bish, 1993, Clays Clay Minerals 41:738

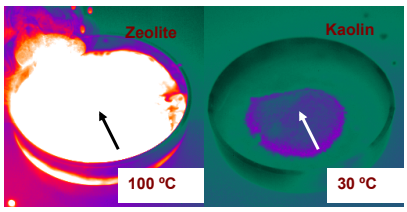
Kaolin vs Dehydrated Zeolite

Thrombelastograph comparison



Clotting Time versus Dosage



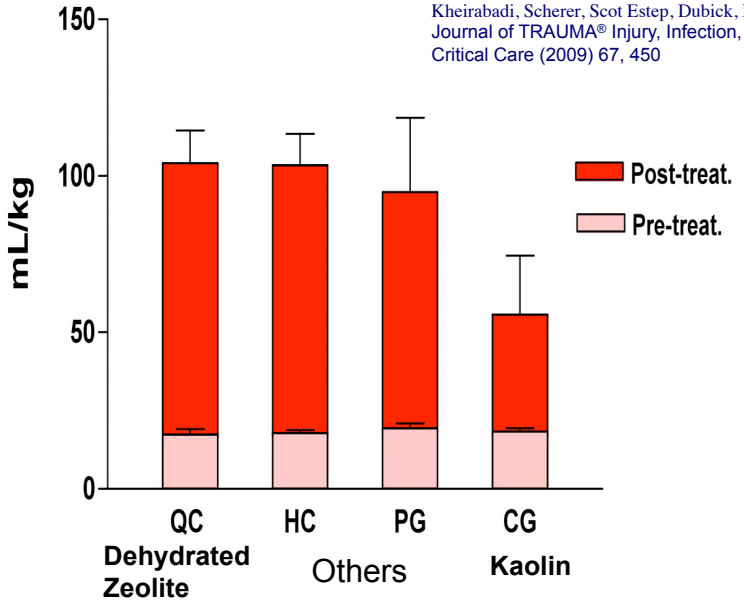


No Heat!

At same or lower dosage.

SE Baker, AM Sawvel, N Zheng, GD Stucky. *Chem. Mater.*, 2007, *19,* 4390

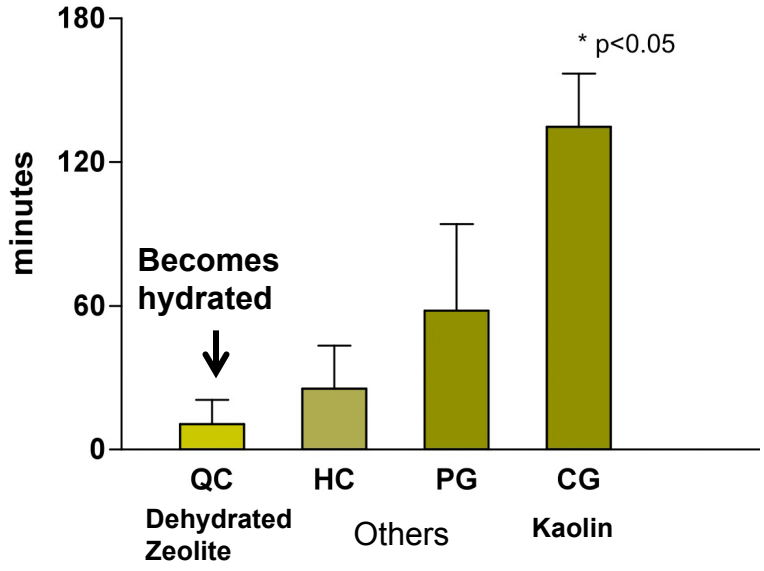
Total Blood Loss



Kheirabadi, Scherer, Scot Estep, Dubick, Holcomb
Journal of TRAUMA® Injury, Infection, and Critical Care (2009) 67, 450

Total HemostasisTime

Kheirabadi, Scherer, Scot Estep, Dubick, Holcomb
Journal of TRAUMA® Injury, Infection, and Critical Care (2009) 67, 450



Current Version

Civilian and Military Use

NORTH AMERICAN RESCUE™
INCORPORATED

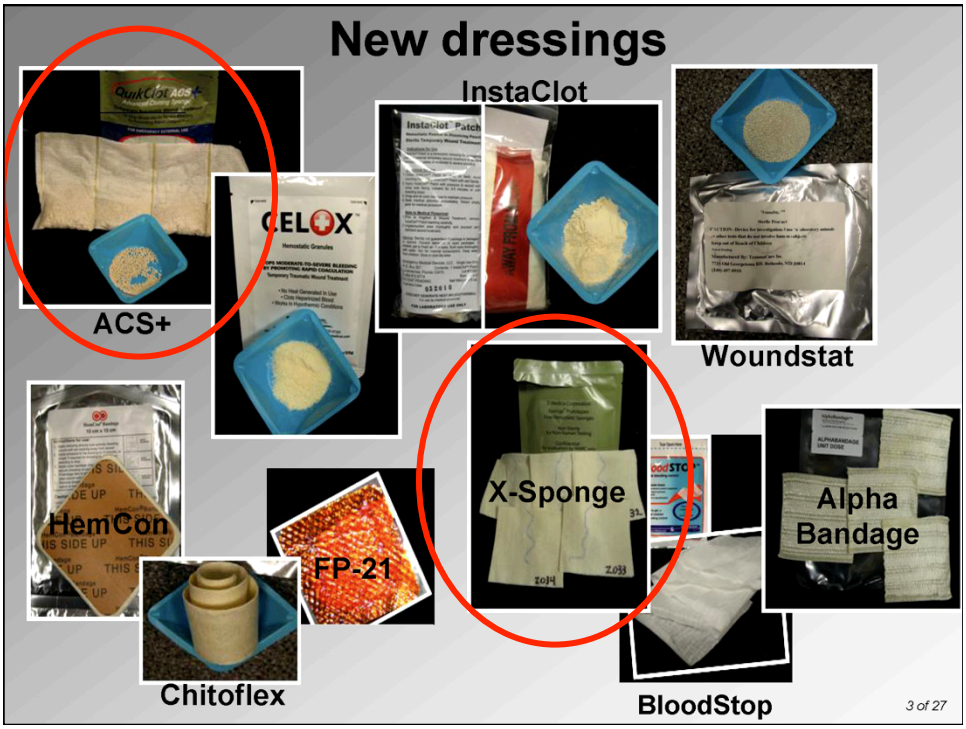
Comparative Testing of Hemostatic Dressings in a Severe Groin Hemorrhage

ATACCC presentation
St Pete, FL 10-13 Aug 2008

Trauma & Resuscitative Medicine Department, NMRC
Silver Spring, MD
Department of Surgery, USUHS
Bethesda, MD

1 of 27

New dressings



3 of 27

"Comparison of new hemostatic granules/powders with currently deployed hemostatic products in a lethal model of extremity arterial hemorrhage in swine" Kheirabadi, et al. J Trauma(2009) 66(2), 316

"Determination of Efficacy of New Hemostatic Dressings in a Model of Extremity Arterial Hemorrhage in Swine" Kheirabadi, Scherer, Estep, Dubick, J. B. Holcomb, *J Trauma* (2009) 67(3) 450

"CG (Kaolin) was the most effective dressing tested in this arterial hemorrhage model. CG is now recommended as the first line of treatment for life-threatening hemorrhage on the battlefield…."

From Iraq to Now (2016)

"Thus far I've trained all 350 of the … soldiers on the quickclot and CAT tourniquet. I cannot begin to express to you how grateful we are for your generosity and kindness. These dressing do make a difference on the battlefield. **Patients that come into the EMT with quickclot in position from early injury have had 100% survival rate**."

Multiple saves reported from many other places

A police officer in Spokane, WA used QuikClot® 1st Response™ brand to save a man after the police officer himself shot the man in the abdomen after the man lunged at him with a knife. Seven saves documented by the Hillsborough County Sheriff's Office in Florida, three in the Hudson Valley of New York, and many more.

With permission, Ray Huey, Z-Medica

U.S. ARMY

GREATEST INVENTIONS 2008 21 September 2009

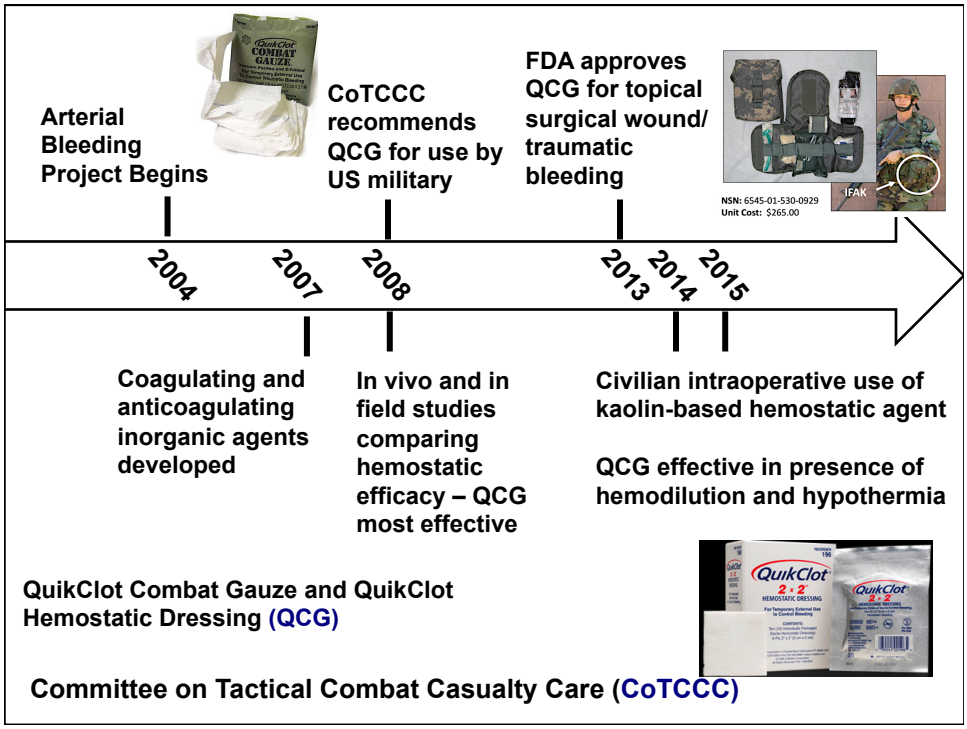
2008 Top Ten Inventions

Dr. Galen Stucky
Invention
Combat Gauze for Treating External Hemorrhages in Injured Soldiers

"CG is now recommended as the first line of treatment for life-threatening hemorrhage on the battlefield…"

U.S. Army Institute of Surgical Research

"Determination of Efficacy of New Hemostatic Dressings in a Model of Extremity Arterial Hemorrhage in Swine" B. S. Kheirabadi et al., *J.* TRAUMA *Injury, Infection, and Critical Care* (2009) 67, 450



A Solution For First Responders

Military Health System Research Symposium, August 2013

- **Field conditions**
  - Austere
  - Limited infrastructure
  - Extreme temperatures and conditions
- **Desired product**
  - Light, portable and deployable
  - Ease of use down to individual Soldier level
  - Example: Combat Gauze carried in each Soldier's Improved First Aid Kit (IFAK) (weighs one pound)

Standard Issue

NSN: 6545-01-530-0929
Unit Cost: $265.00

NSN	NOMENCLATURE	UNIT PACK QTY
8465-01-531-3647	100 Round SAW/Utility Pouch, MOLLE II	1
6515-01-521-7976	Tourniquet, Combat Application	1
6510-01-492-2275	Bandage Kit, Elastic	1
	OR	
6510-01-460-0849	Bandage Kit, Elastic	1
6510-01-503-2117	Bandage GA4-1/2" 100's	1
6510-00-926-8883	Adhesive Tape Surg 2" 6's PG	1
6515-01-180-0467	Airway, Nasopharyngeal	1
6515-01-519-9161	Glove, Patient Exam 100's	4
6545-01-586-7691	Contents Kit, IFAK Resupply Kit	1
6545-01-531-3147	Insert (folding panels with cord)	1
6510-01-562-3225	Dressing, Combat Gauze	1*

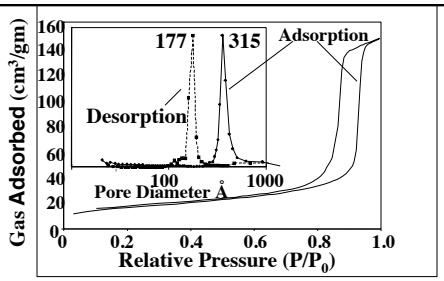
*The Combat Gauze has a 36-month shelf life, so it is shipped separately.

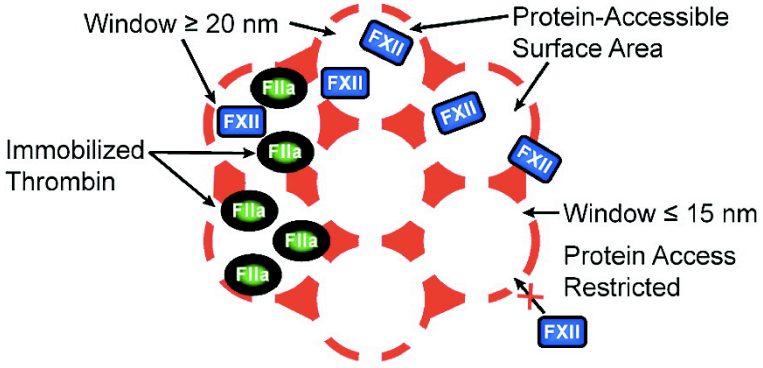
QCG Remains Hemostatic Choice in 2014

- **QuikClot Combat Gauze® (QCG) reaffirmed by Committee on Tactical Combat Casualty Care (CoTCCC) as hemostatic dressing of choice**
- **More than 5 million units of QCG shipped to five branches of US Military by Z-Medica**
- **No product-related adverse reactions reported**

A better performing candidate

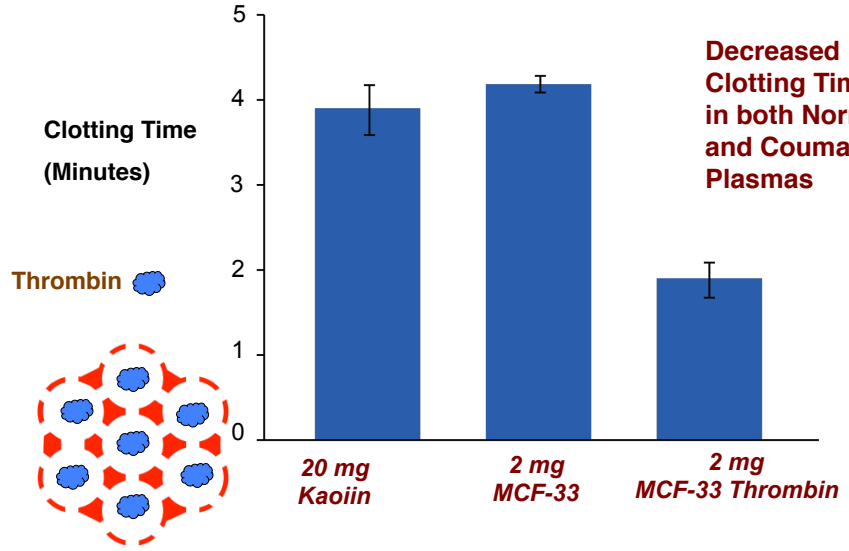
Mesocellular Foam





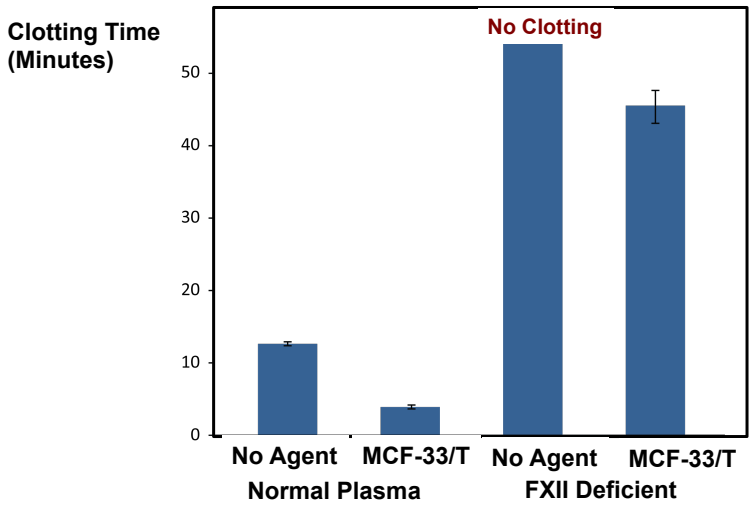
Schmidt-Winkel, et al *J. Amer. Chem. Soc. 121*, 254 (1999)

Clotting time MCF-33 ≈ Combat Gauze at a significantly lower dose



Baker, S.E., Sawvel, A.M., et al., *Langmuir* 2008, *24,* 14254

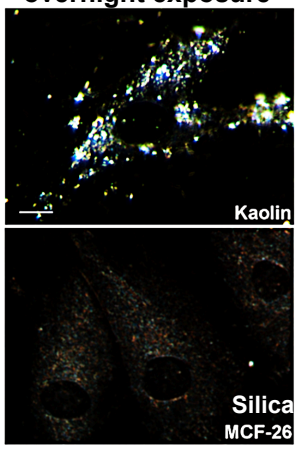
MCF @ thrombin activates clotting through the contact pathway



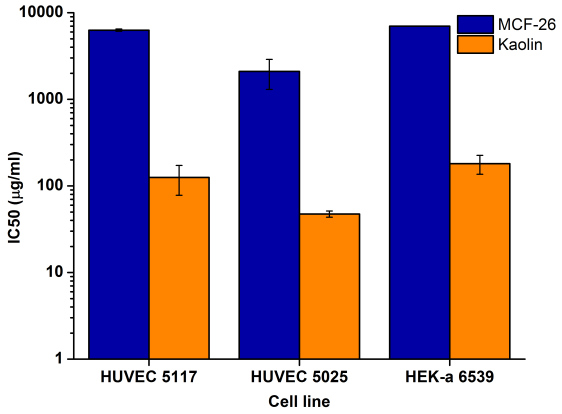
Baker, S.E.*et al.*, *Langmuir* 2008, *24,* 14254

Silica-based particles (MCF) are less cytotoxic than aluminosilicates (zeolites and kaolin)

Cellular uptake after overnight exposure

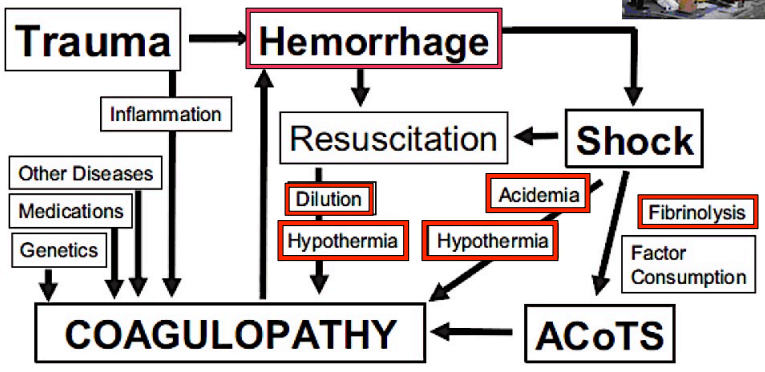


IC50 was determined after overnight exposure of the cells to mesoporous silica and kaolin.



Li, Y, Sawvel, A M, Jun, Y, et al. *Toxicology Research* 2013, 2(2), 136

Blunt Trauma Trajectory -- Internal bleeding & long time line



Coagulopathy : Blood's ability to clot is impaired

Genetic: How well does your body regulate clotting-anticlotting?

J. R. Hess, et a*l*, The journal of **TRAUMA** Injury, Infection, and Critical Care, 2008

D. Frith, Mitchell J. Cohen, K. Brohi, Thrombosis Research 129 (2012) 551-556

Coagulopathy

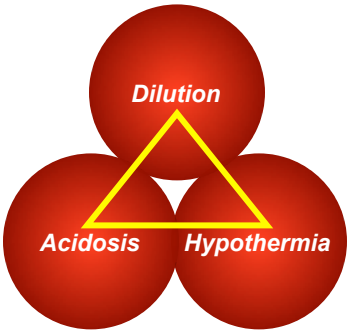
Fundamental breakdown of the human coagulation cascade system

"Trauma Triad of Death"

Inability to Maintain Normal Hemostasis

Need early predictors of mortality – fast therapeutic response decisions

anaerobic metabolism & lactic acid production →



Factors That Influence Traumatic Coagulopathy

ACoTS: Acute Coagulolpathy fromTraumatic Shock

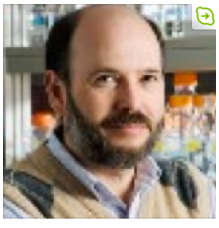
Parr, Michael J. et al. *J. Trauma*, 2008, *65*, 766

Dickneite, Gerhard. et al. *Anesthesia & Analgesia*, 2008, *106*, 1070

Threshold switchable particles for control of internal hemorrhage

Stephanie A. Smith DVM MS
University of Illinois College of Medicine

James H. Morrissey
Molecular & Cellular Biology
Department of Biochemistry
University of Illinois



Rustem Ismagilov
California Institute of Technology

Ying Liu
University of Illinois Chicago

Damien Kudela
Chi Nguyen
Anna May-Masnou
Alessia Pallaoro
Tracy Chuong
Scott Hammond
Galen Stucky
Gary B. Braun
Erkki Ruoslahti
UCSB & Burnham Institute for Medical Research

Christian Kastrup
University of British Columbia

For Internal Bleeding Cardiovascular Targeting and Delivery

Thrombin or prothrombin are strong coagulating agents – what about

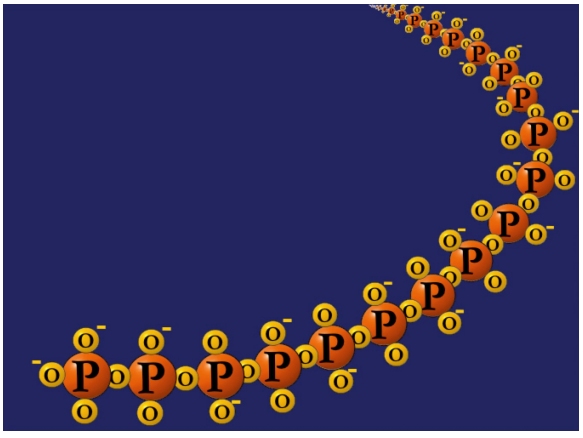
- **Thrombosis**
- **Biodistribution ??**

WHAT IF WE HAD

Threshold switchable particles for the control of internal hemorrhage?

No need for packaging or encapsulation !

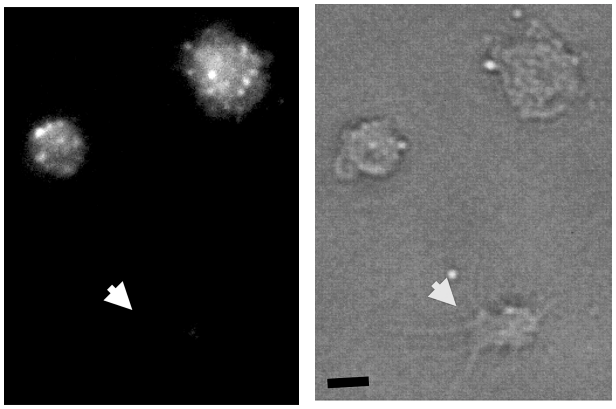
Polyphosphate (PolyP)



Linear polymer of phosphate units

Stephanie A. Smith, James H. Morrissey

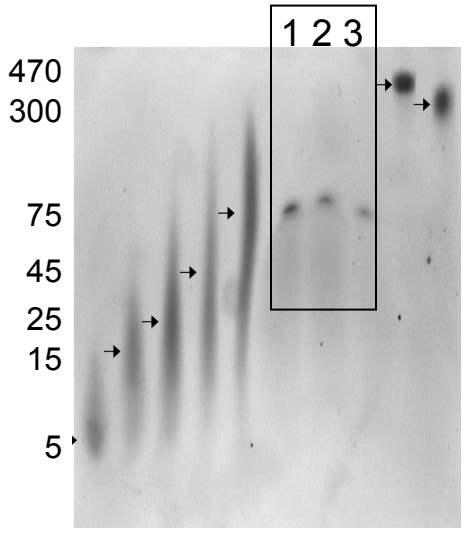
Polyphosphate in Platelets



PolyP secreted by platelets in response to thrombin stimulation

Ruiz FA, Lea CR, Oldfield E, Docampo R.
Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes
J Biol Chem 2004; 279(43):42250-57

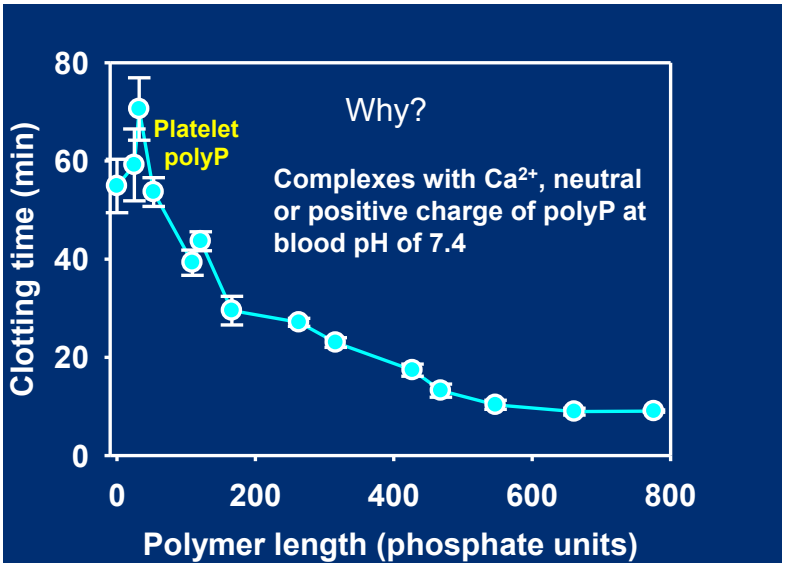
Polyphosphate in Platelets



P50 to P75

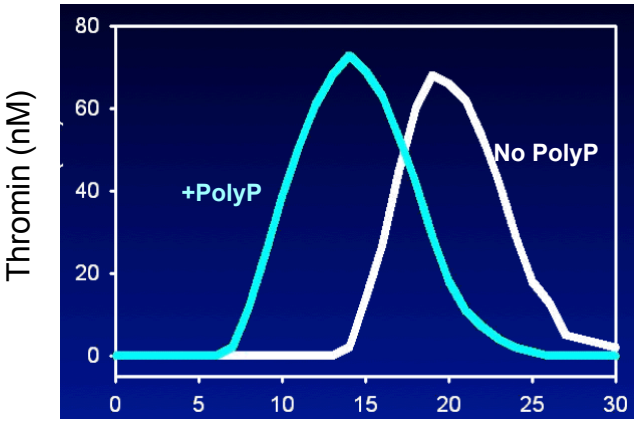
Ruiz FA, Lea CR, Oldfield E, Docampo R.
J Biol Chem 2004; 279(43):42250-57

Platelet polyP is a poor coagulator for normal blood



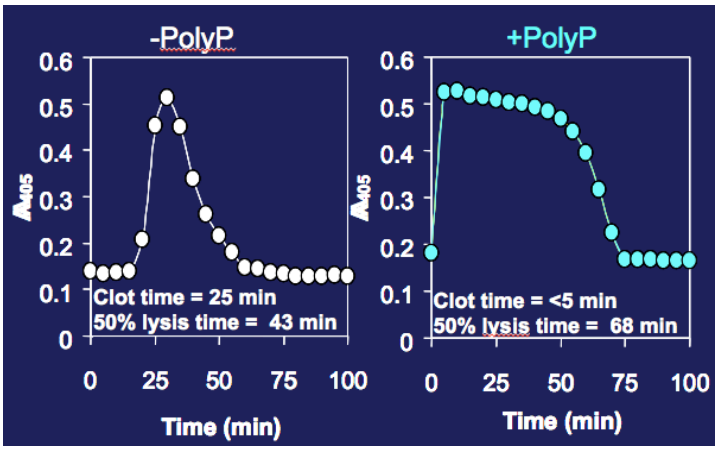
Smith, S. A. *et al.* Polyphosphate exerts differential effects on blood clotting, depending on polymer size. *Blood* 116, 4353 (2010).

Thrombin Generation with Tissue Factor Added



Smith, Morrissey

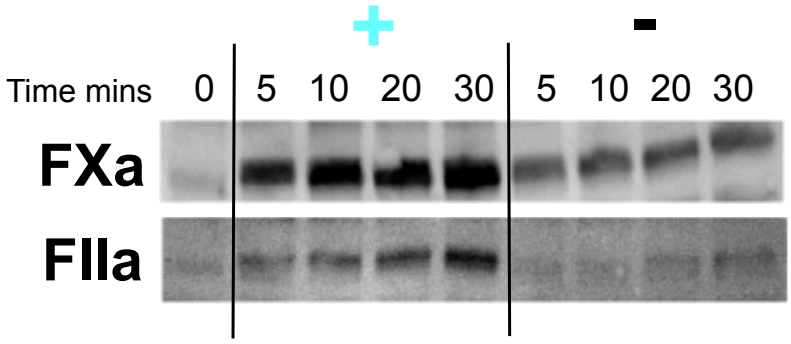
Coagulation and reduced fibrinolysis with PolyP



Smith, Morrissey

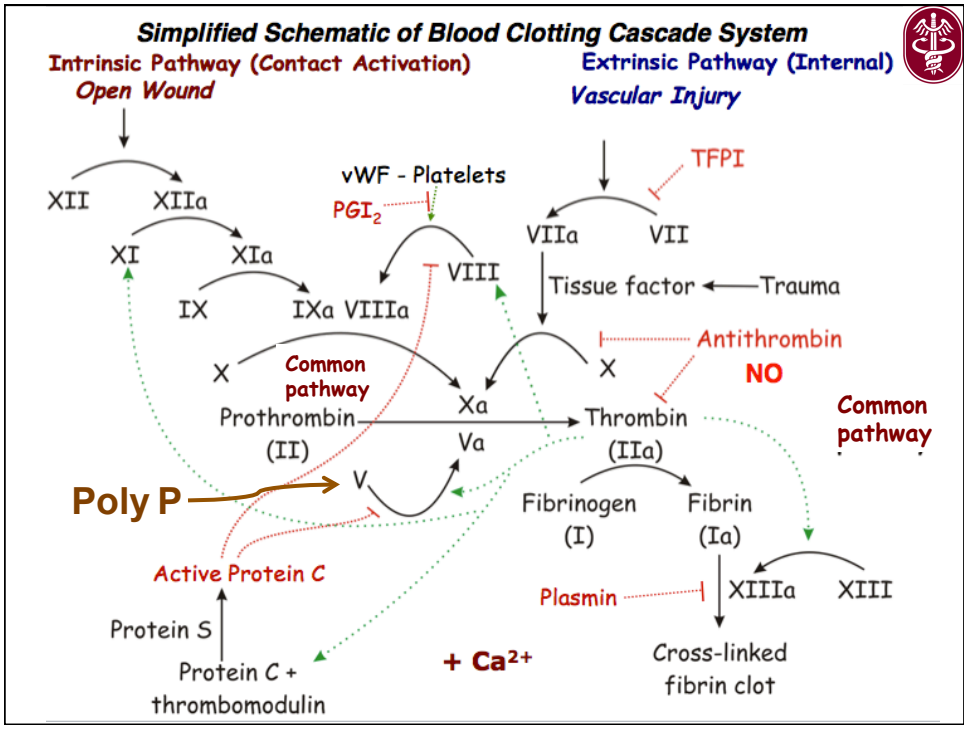
FV Activation

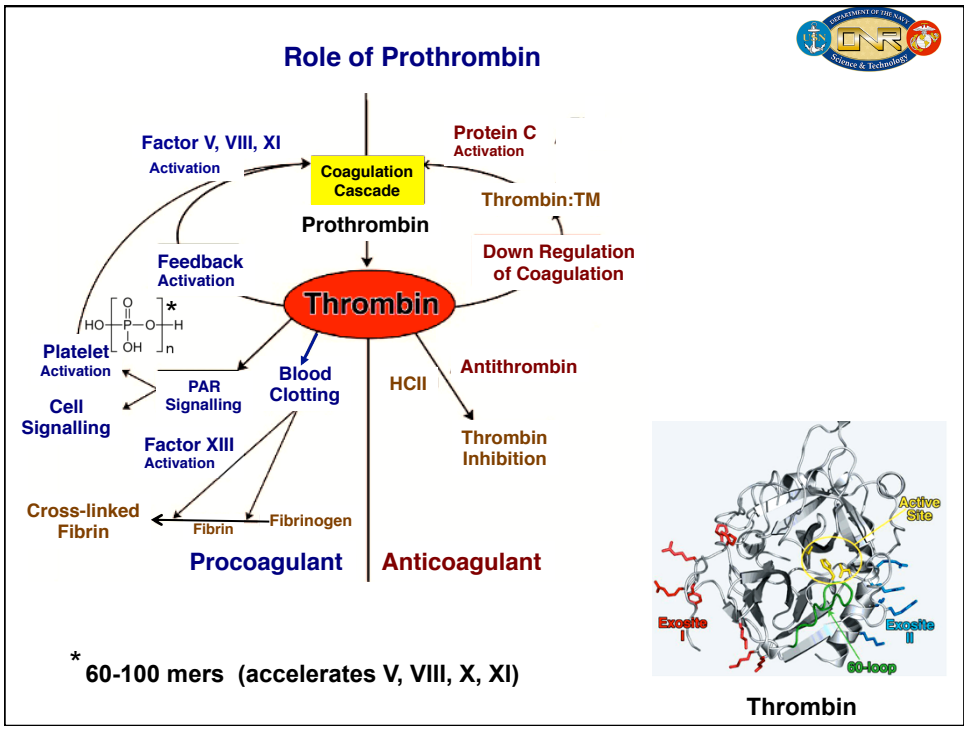
PolyP enhances the rate of cleavage of FV to FVa by both FIIa & FXa

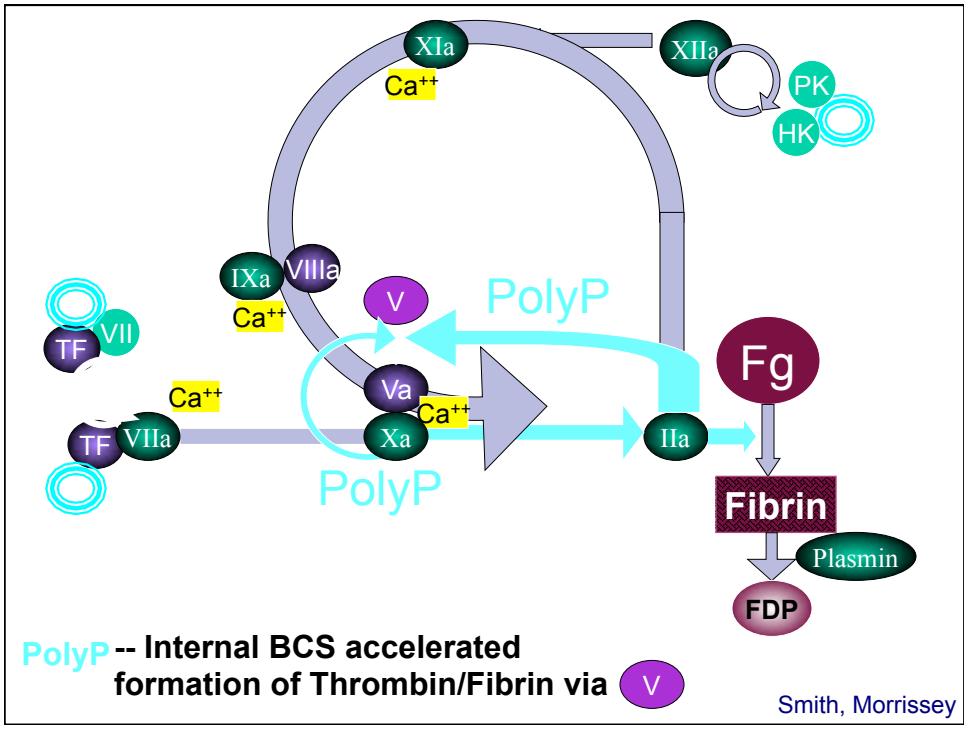


FVa heavy chain
Indicates cleavage of Fv to FVa

Smith, Morrissey

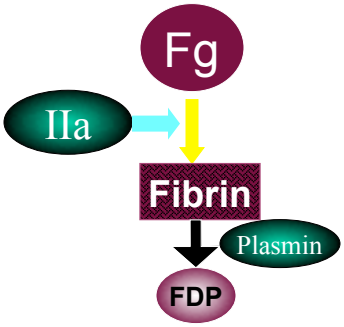

Simplified Schematic of Blood Clotting Cascade System
Intrinsic Pathway (Contact Activation)
Open Wound
Extrinsic Pathway (Internal)
Vascular Injury
XII
XIIa
XI
XIa
IX
IXa
vWF - Platelets
PGI2
VIII
VIIIa
TFPI
VIIa
VII
Tissue factor
Trauma
Antithrombin
NO
X
Common pathway
Prothrombin (II)
Xa
Va
Thrombin (IIa)
Common pathway
Poly P
V
Fibrinogen (I)
Fibrin (Ia)
Active Protein C
Plasmin
XIIIa
XIII
Protein S
Protein C + thrombomodulin
+ Ca2+
Cross-linked fibrin clot


Role of Prothrombin
Factor V, VIII, XI
Activation
Coagulation Cascade
Protein C
Activation
Thrombin:TM
Prothrombin
Down Regulation of Coagulation
Feedback
Activation
Thrombin
Platelet
Activation
Cell Signalling
PAR Signalling
Blood Clotting
HCII
Antithrombin
Thrombin Inhibition
Factor XIII
Activation
Cross-linked Fibrin
Fibrin
Fibrinogen
Procoagulant
Anticoagulant
* 60-100 mers (accelerates V, VIII, X, XI)
Active Site
Exosite I
Exosite II
60-loop
Thrombin

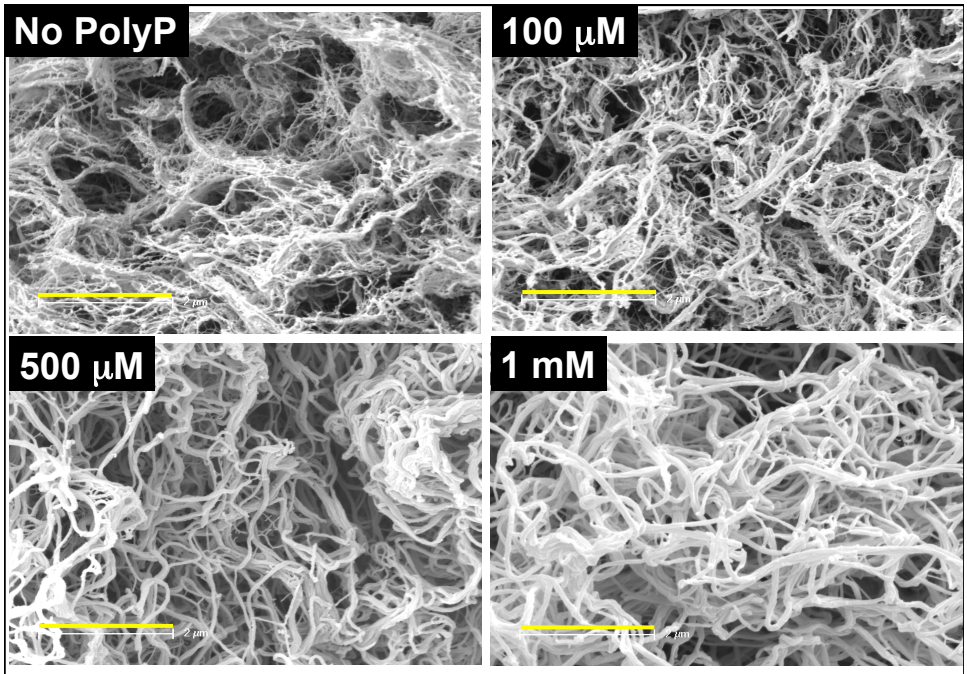


What else?

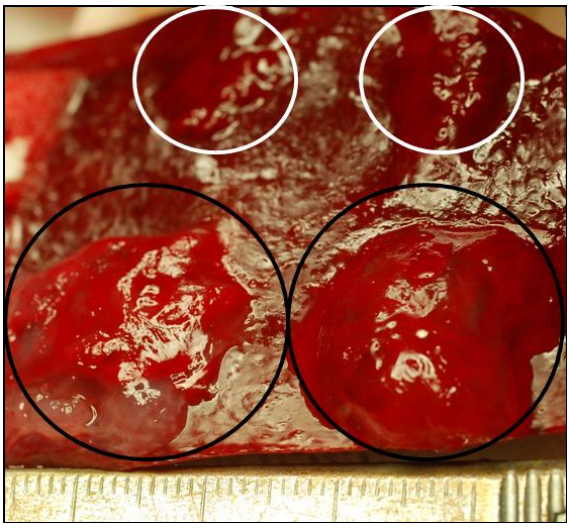
Effect of polyP on clot structure



Smith, Morrissey



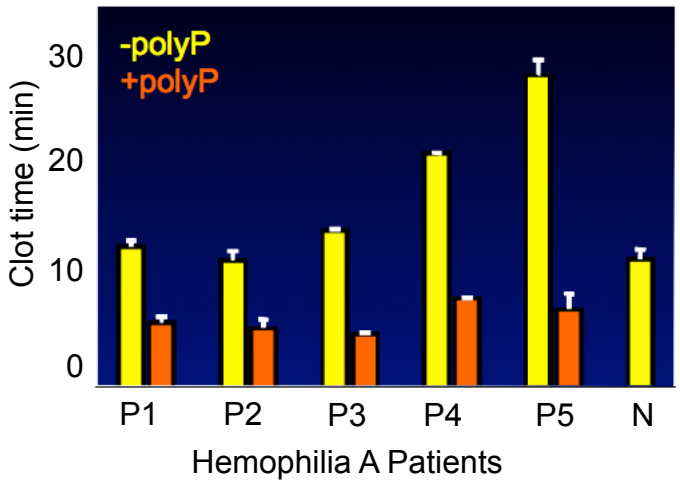
Fibrin Tissue Sealant



No PolyP

+ PolyP

Shortens Clot Time in Hemophilia A



➢ **Shortens Clot Time with Coumadin or Heparin Therapy**

Smith, Morrissey

How can we make point-of-care & clinical use of PolyP ?

- As an accelerator of blood clotting cascade system **at trauma sites only**
- **As a means of defining the trauma trajectory to minimize "lethal triad" and coagulopathy**

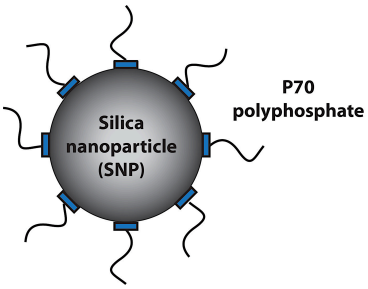
Strategy for functionalization: targeting & protection

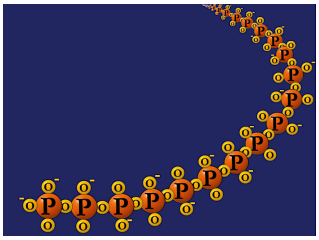
electrostatic – hydrogen bonding

SiO_2 NP's

pK_a 7.6 to 8.4

pK_b 1.9



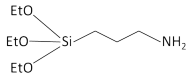


Phosphates	pK_a
orthophosphate	2.15, 7.20, 12.35
pyrophosphate	0.8, 2.2, 6.7, 9.4
tri(poly)phosphate	0.5, 1.0, 2.4, 6.5, 9.4

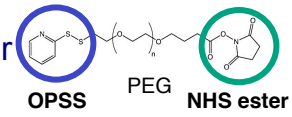
Kudela, Masnou, Stucky

Strategy for functionalization: targeting & protection

1. Amino-group



2. Linker

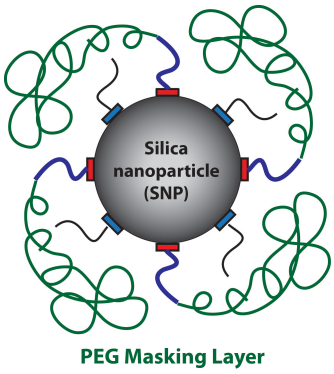


3. Peptide

4. PEG

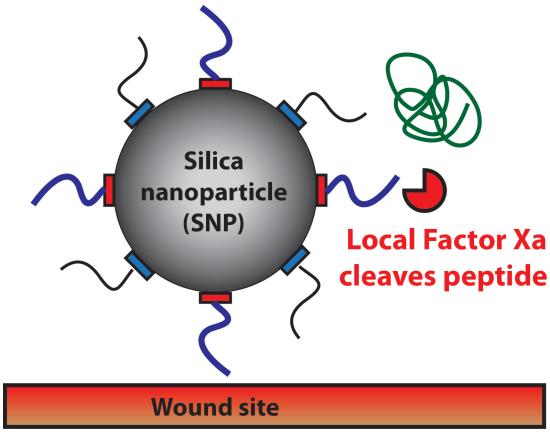
(2k, 5k, 20k)

Covalent functionalization



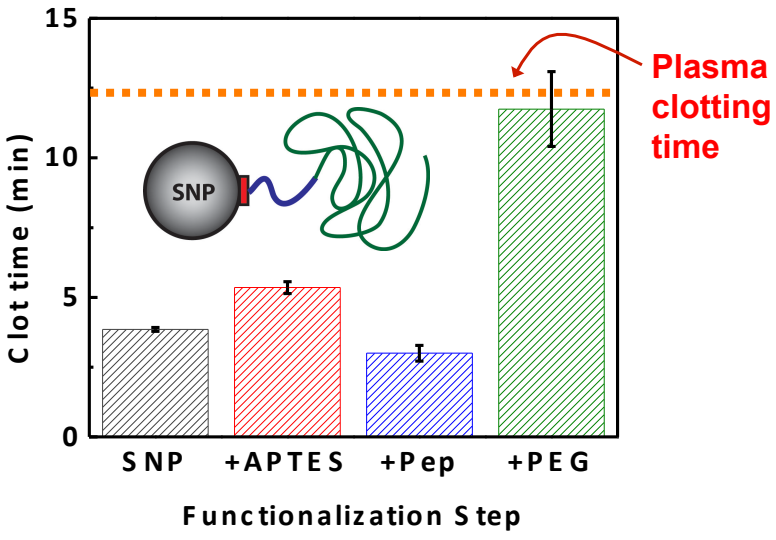
Nguyen, Kudela, Masnou, Stucky

Strategy for functionalization: targeting & protection



Nguyen, Kudela, Masnou, Stucky

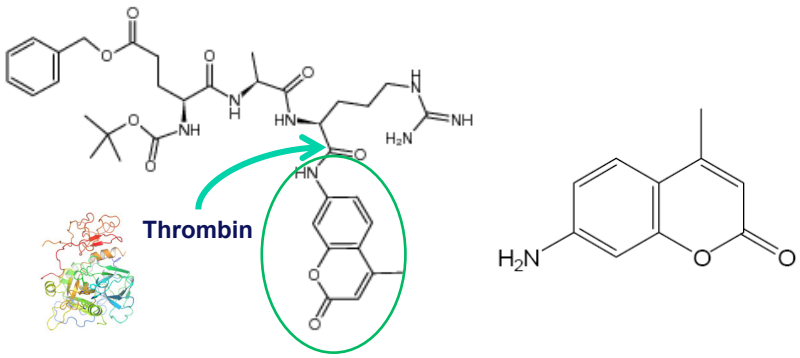
Steps of functionalization: clot time increases with PEG



Nguyen, Kudela, Masnou, Stucky

Thrombin generation: plate reader

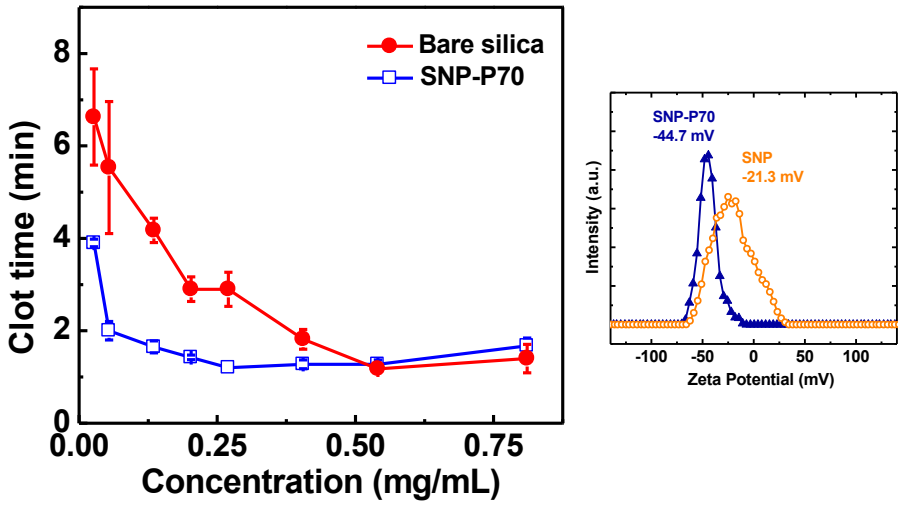
Thrombin-sensitive dye Boc-Asp(OBzl)-Pro-Arg-MCA



- Coumarin derivative C120 exhibits fluorescence

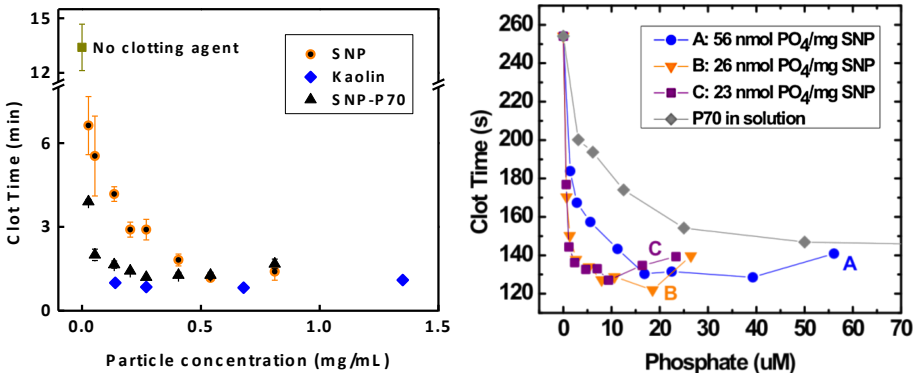
Kudela, Masnou

Polyphosphate-coated silica nanoparticles (SNP-P70) have lower clotting time than SNP

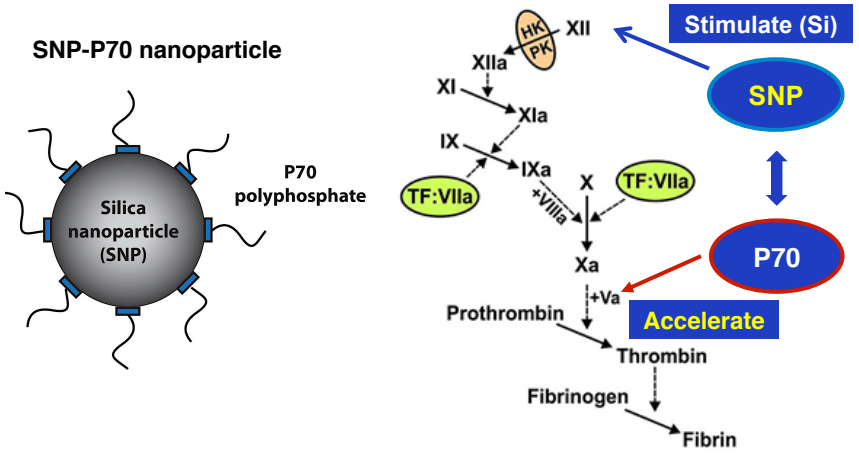


Kudela, Masnou

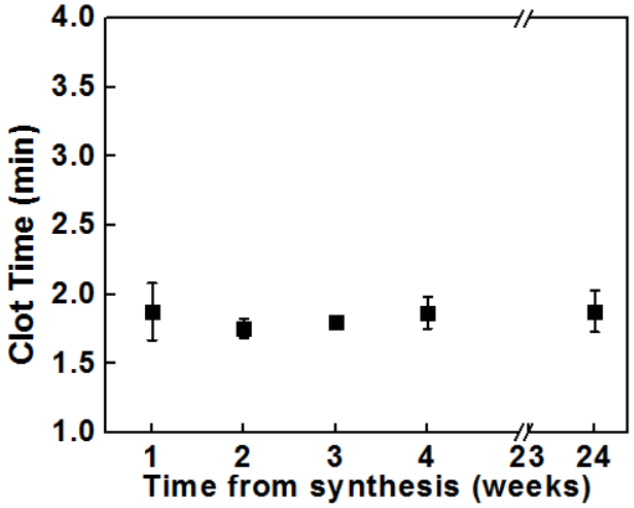
Polyphosphate coated silica nanoparticles (SNP-P70) are similar to kaolin and better than polyP in solution



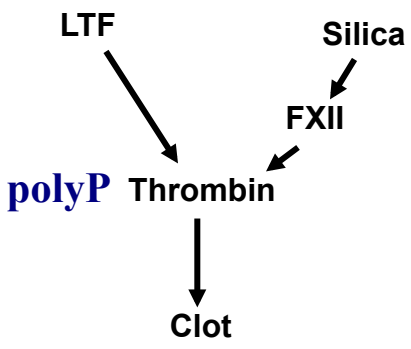
SNP-P70 stimulates and accelerates clotting: synergistic effect

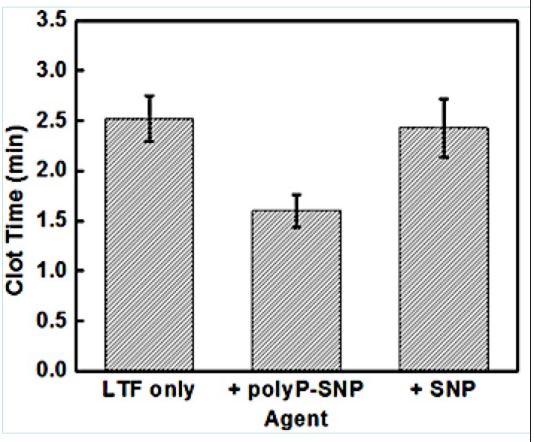


PolyP-SNP is stable at ambient conditions



PolyP promotes coagulation in FXII deficient plasma





Kudela D, et al. Clotting activity of polyphosphate-functionalized silica nanoparticles. *Angew Chem Int Ed Engl*. 2015;54(13):4018

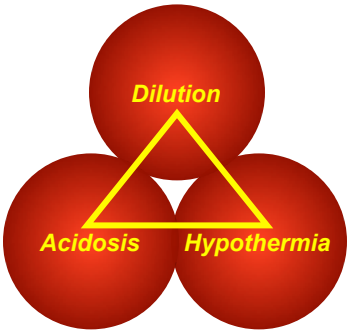
Coagulopathy - The fundamental breakdown of the human coagulation cascade system

"Trauma Triad of Death"

Inability to Maintain Normal Hemostasis

Need early predictors of mortality – fast therapeutic response decisions

anaerobic metabolism
& lactic acid production →



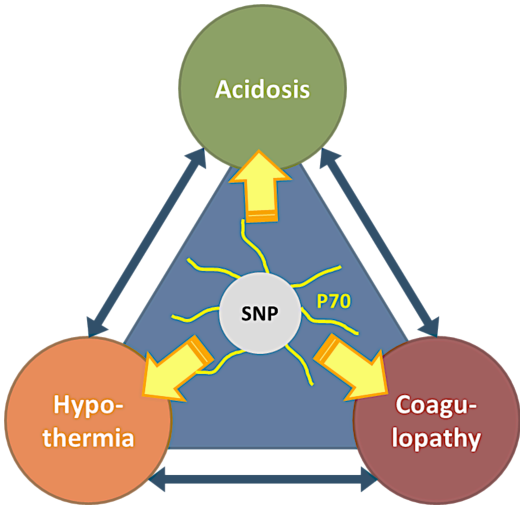
Factors That Influence Traumatic Coagulopathy

ACoTS: Acute Coagulopathy from Traumatic Shock

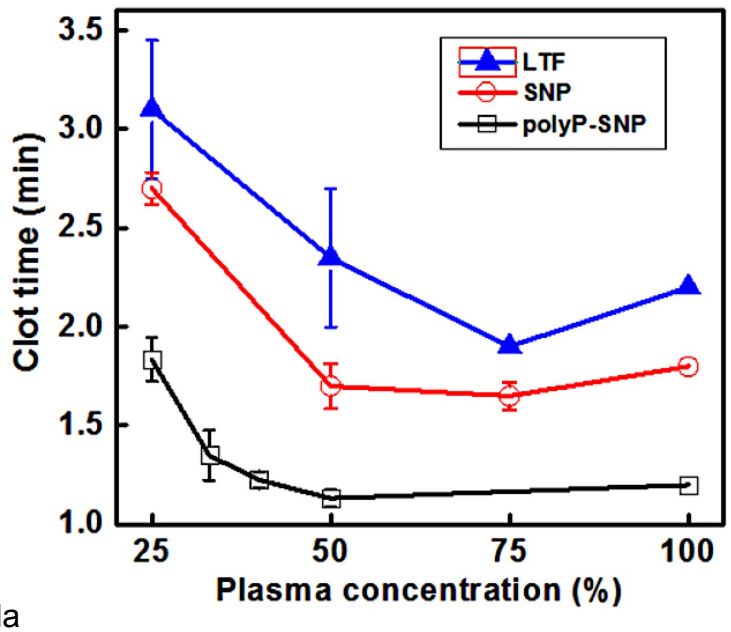
Parr, Michael J. et al. *J. Trauma*, 2008, *65*, 766

Dickneite, Gerhard. et al. *Anesthesia & Analgesia*, 2008, *106*, 1070

Testing the nanoparticles under coagulopathic conditions: the "lethal triad" trauma trajectory

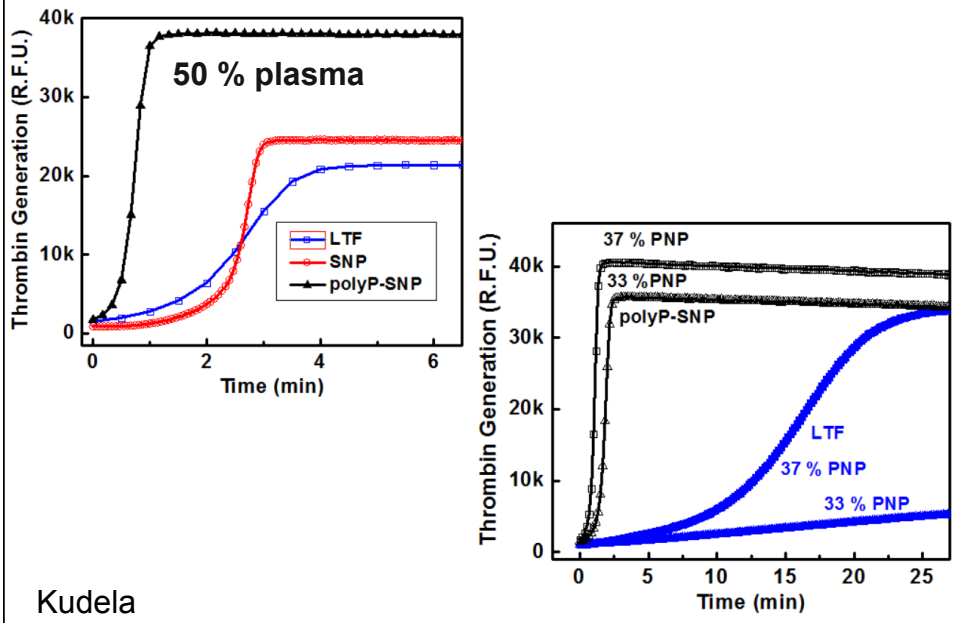


Hemodilution: TEG results



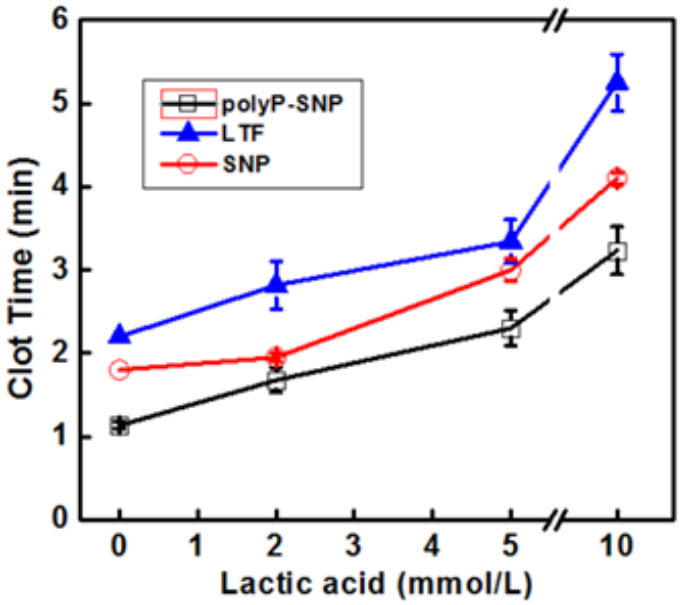
Kudela

Hemodilution – Thrombin Production

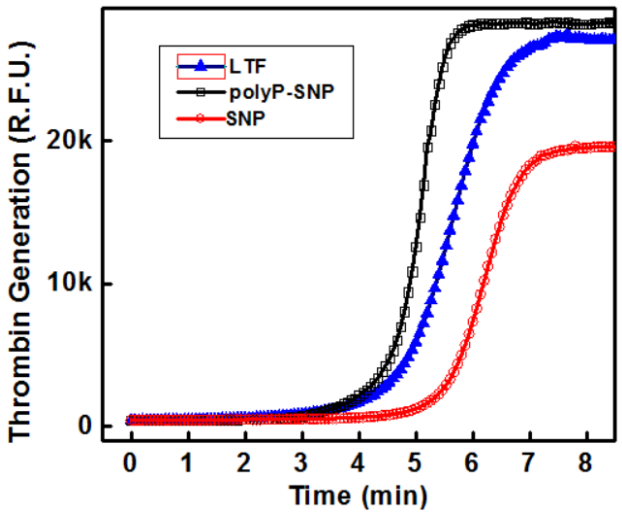


Kudela

Acidosis – TEG

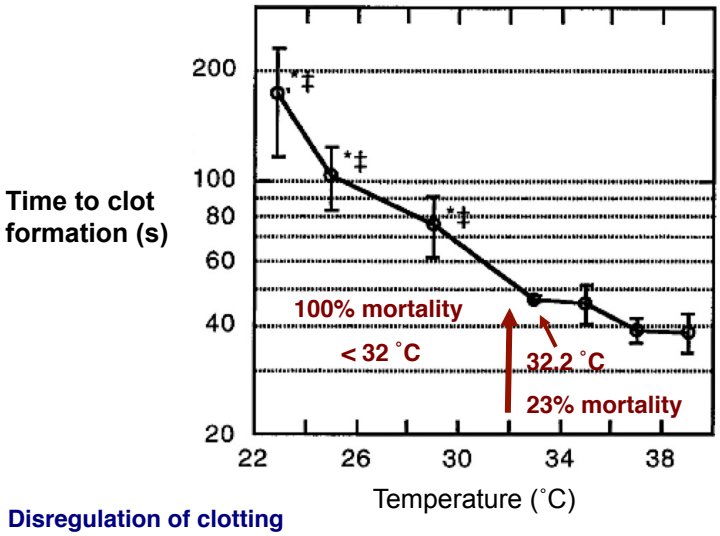


Acidosis – Thrombin Production



Hemostasis & Temperature

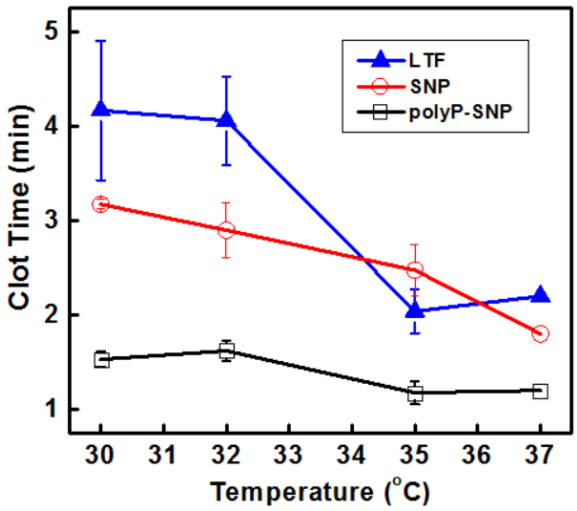
Hypothermia induced coagulopathy?



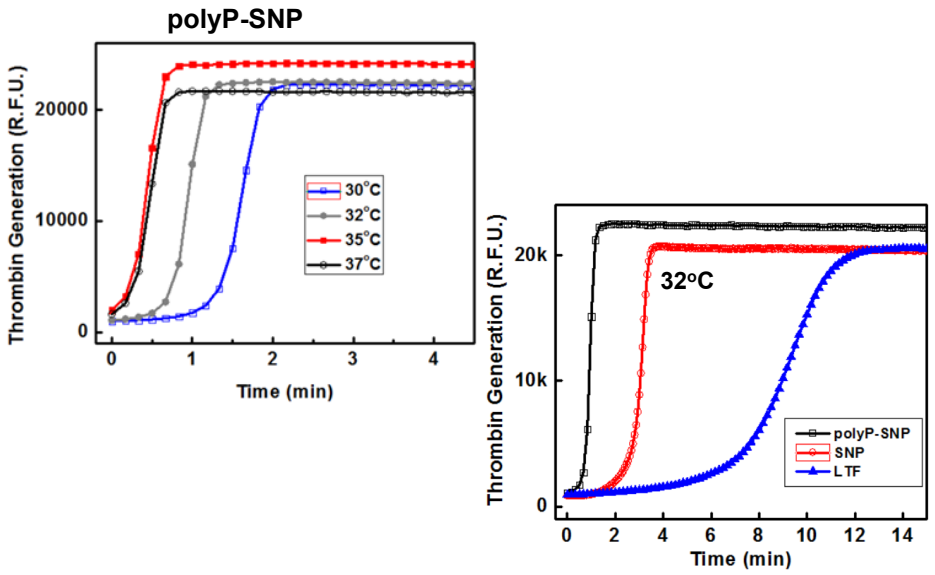
Disregulation of clotting factors and platelets

Wolberg, A S, et. al. J. Trauma (2004) 56, 1221

Hypothermia - TEG results



Hypothermia –thrombin production



Take home message

Inside BCS therapeutic with PP triggering	**Active Ingredients: Polyphosphate & pro- or non-coagulating carrier (e.g. SiO_2 or hydroxyapatite)**
Biocompatibie	**Uses materials that minimize harmful side effects**
Directs trauma trajectory away from coagulopathy	**Expands dilution, hypothermia, acidemia windows**
Universal use	**Can be modified to treat external and internal bleeding**

Translating polyP-SNP into clinical use

Proof of concept
Safety, efficacy

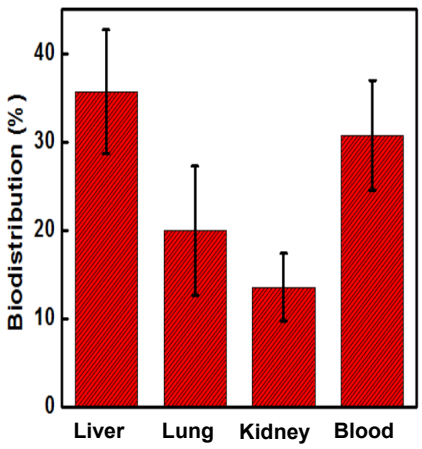
Complex model
Efficacy
Safety
FDA IND

Clinical Trials

Collaboration with Chi Nguyen (Stucky lab) and Kyle Ploense (Kippin lab)

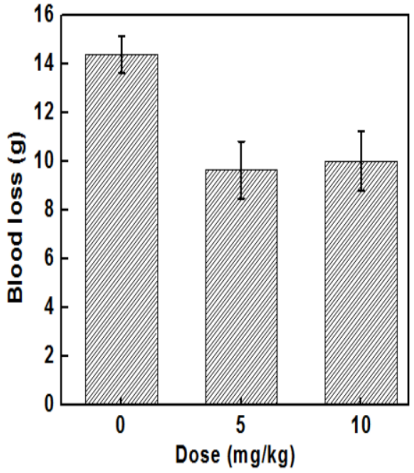
Safety studies show no thrombosis

Histopathology Reports (Charles River): no thrombi, no **clear** microthrombi, no vascular changes, and no necrosis.

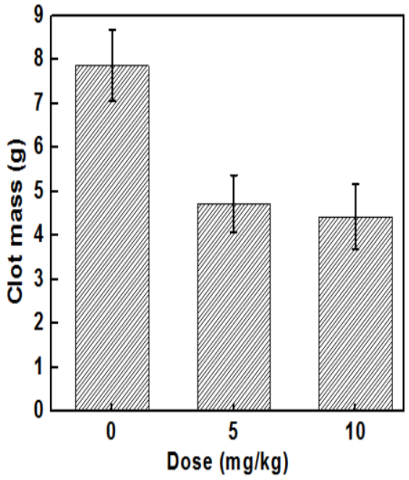


ICP-MS conducted by Chi Nguyen (Stucky lab) in collaboration with Kyle Ploense (Kippin lab)

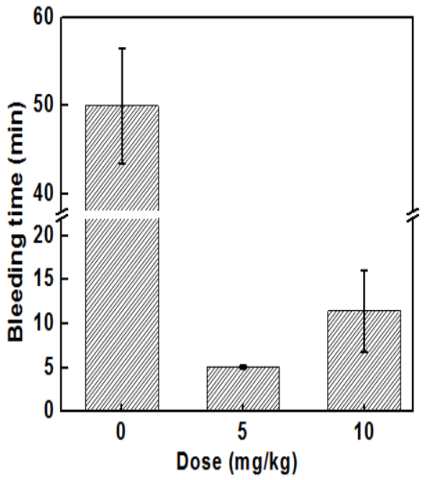
PolyP-SNP reduces blood loss 33 % after tail injury (p = 0.0097)



PolyP-SNP reduces overall clot size by forming smaller, denser clots directly at the wound site (p = 0.074)



PolyP-SNP quickly stops all blood loss (p = 0.0005)



The Challenge and Where We Are

- ✓ ♦ **Open wound, rapid response treatment --- in the field**
  - ✓ **Stop arterial hemorrhaging in < 5 minutes by interfacing the blood clotting system with an externally applied agent**
- ✓ ♦ **Internal bleeding -trauma trajectory - create a longer time window for 1st responders and clinicians (*in vitro*)**
  - ✓ **Lethal Triad ⟷ Hypothermia – Acidosis – Dilution**
- ✓ ◆**Traumatic Coagulopathy – can accelerate total blood clotting system with targeted, non-procoagulating, therapeutic delivery (*in vitro*)**

Office of Naval Research

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What Next?

Translation of polyP-SNP into clinical use

UCSB –Cayuga Inc.
Synthesis polyP-SNP
In vitro clotting assays
Adapt polyP-SNP preclinical and clinical trials

Small mammal studies
UCSF (Cohen lab), UCSB
In vivo proof of concept

Clinical
Use for trauma patients
(UCSF, Davis, Irvine Huntington Medical Research)

Preclinical large mammal studies
Primates/swine at UC Davis – UC Irvine
Travis AFB
Preclinical (human use)

Thanks!