Current testing and alternative methods for assessment of medical device blood-material interactions

Society of Toxicology
Medical Device Specialty Section

Michael F. Wolf
Science, Technology, and New Therapies
Medtronic Inc.
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"The dose makes the poison"

- Paracelsus view of chemicals

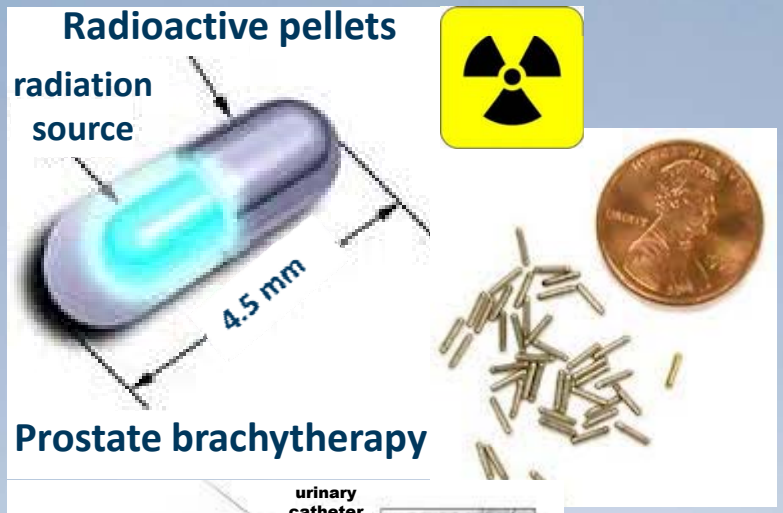
Medieval Definition of Biocompatibility

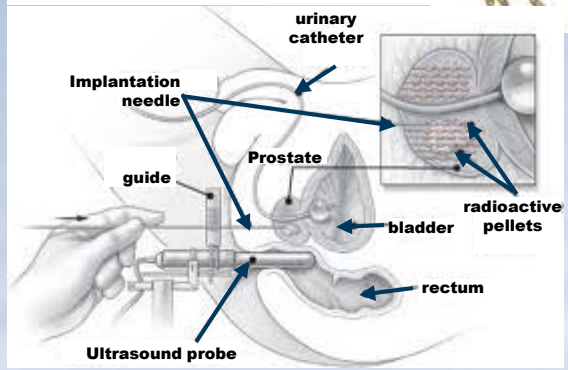
Implantable Metal
(not biocompatible)

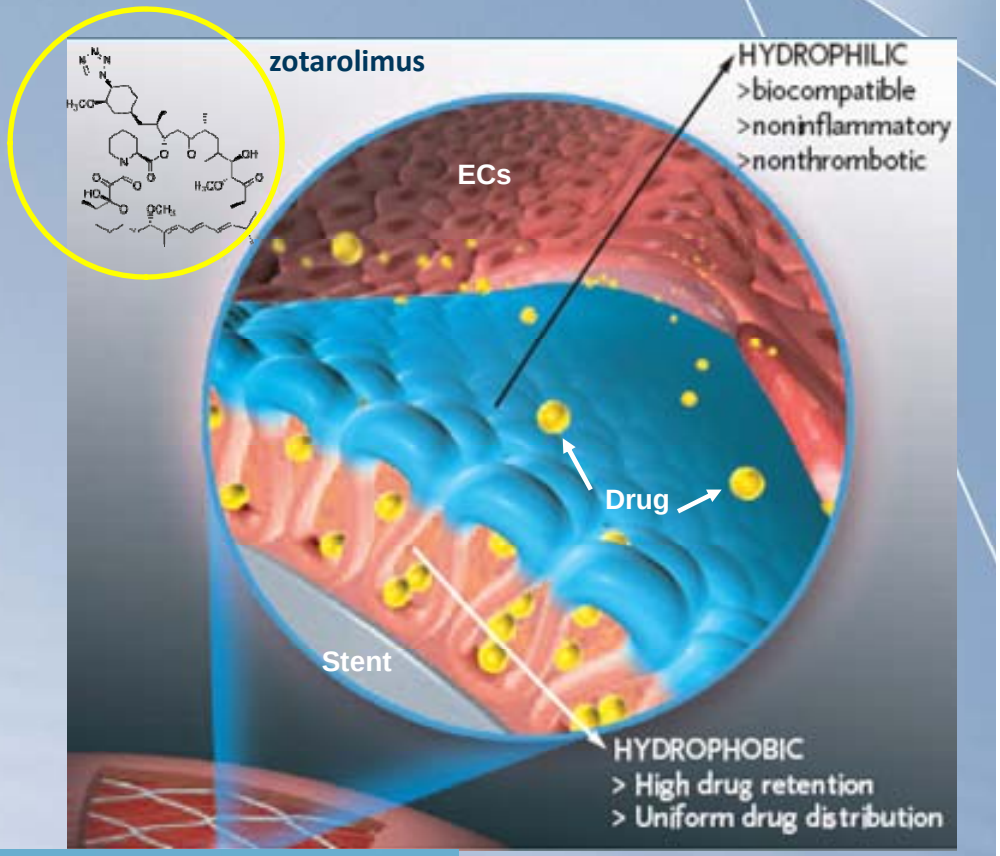
Biocompatible Metal
(not implantable)

"The dose makes the poison"

- for some medical devices







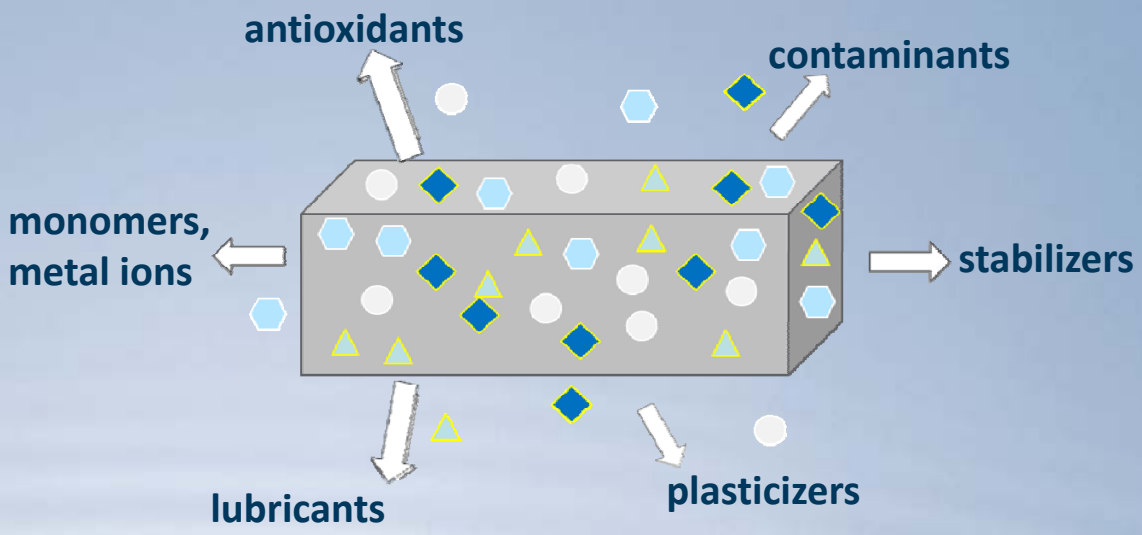
Drug Eluting Stents

For most medical devices
the "poison" (degree of biocompatibility) made by:

- **the material(s)**
- **the material surface area**
- **type of tissue contact, and contact time**
- **the device design**
- **other factors (patient, physician, etc..)**

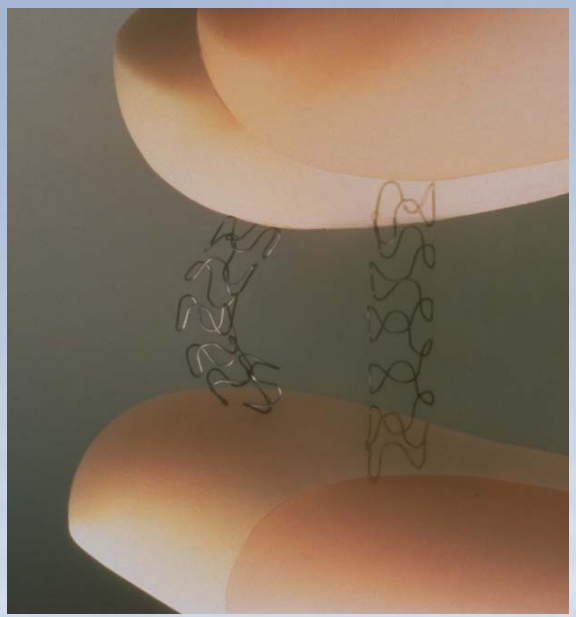
For most medical devices
the “poison” (degree of biocompatibility) made in part by:

- **the material(s)**



For most medical devices
the “poison” (degree of biocompatibility) made in part by:

- **the material surface area (SA)**



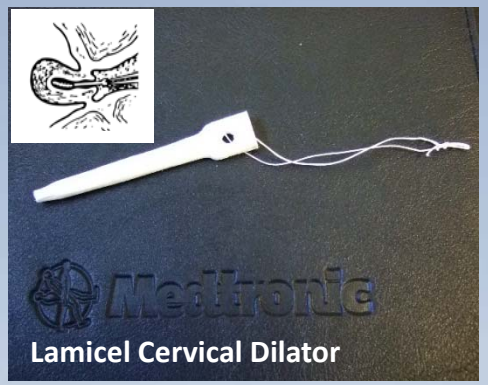
Coronary stent
surface area (SA) ≈ 1 cm^2

CPB blood oxygenator
surface area (SA) ≈ 25,000 cm^2

For most medical devices the "poison" (degree of biocompatibility) made in part by:

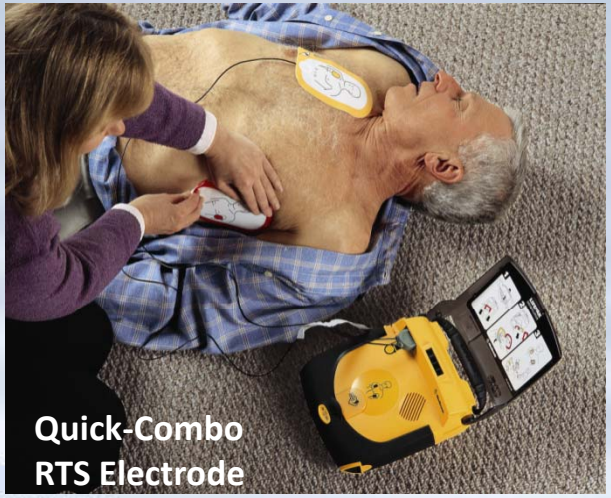
- **type of tissue contact and contact time**

Sensors and Infusion Sets

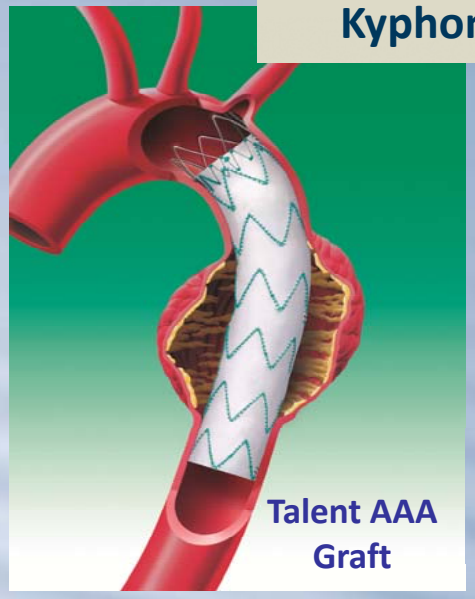


Lamicel Cervical Dilator

Kyphon Balloon



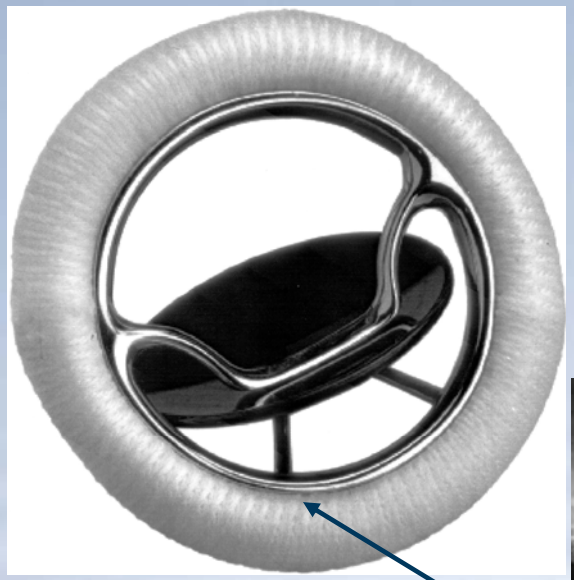
Quick-Combo RTS Electrode



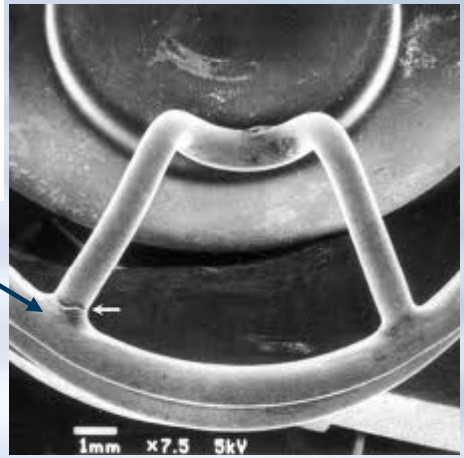
Talent AAA Graft

For most medical devices the "poison" (degree of biocompatibility) made in part by:

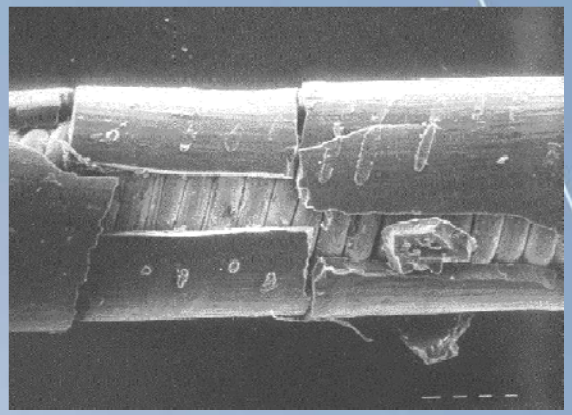
- **the device design**

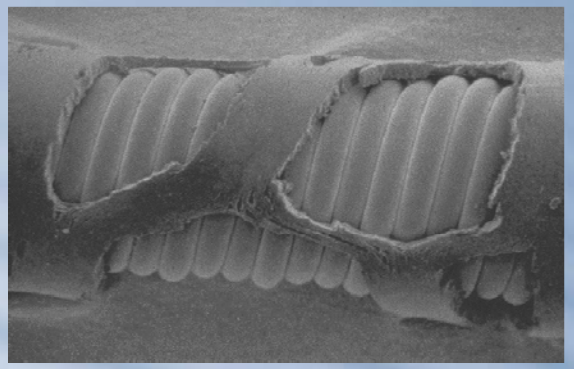


Heart valve strut



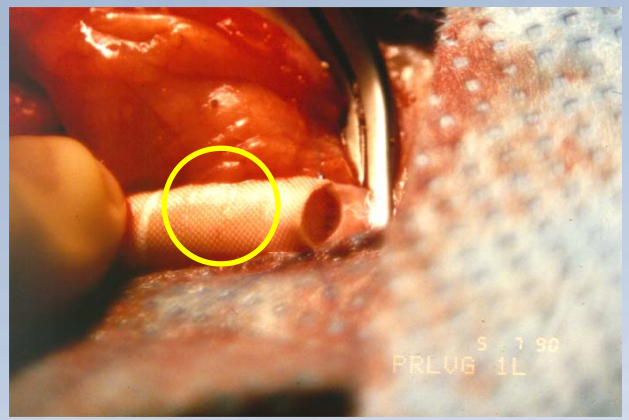
Pacing lead insulation

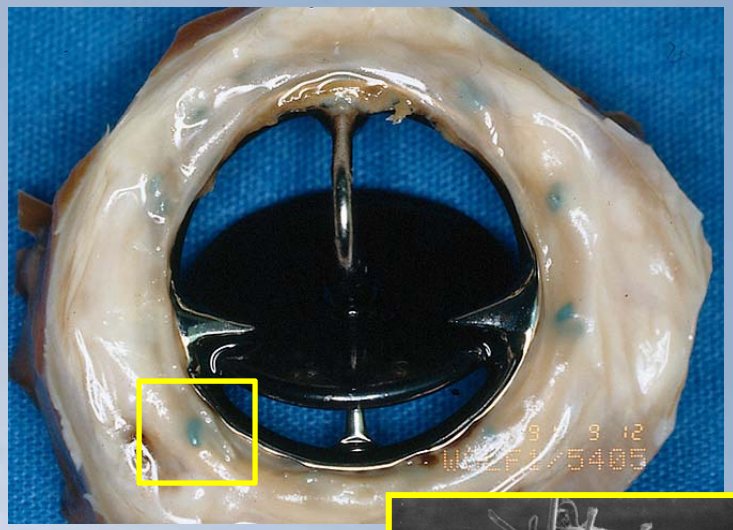


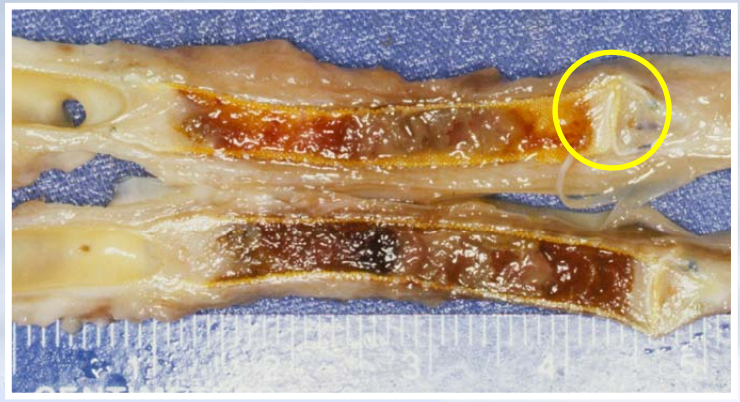


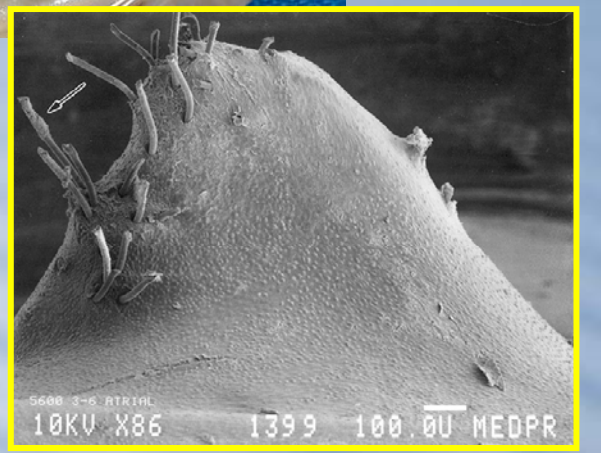
For most medical devices
the “poison” (degree of biocompatibility) made in part by:

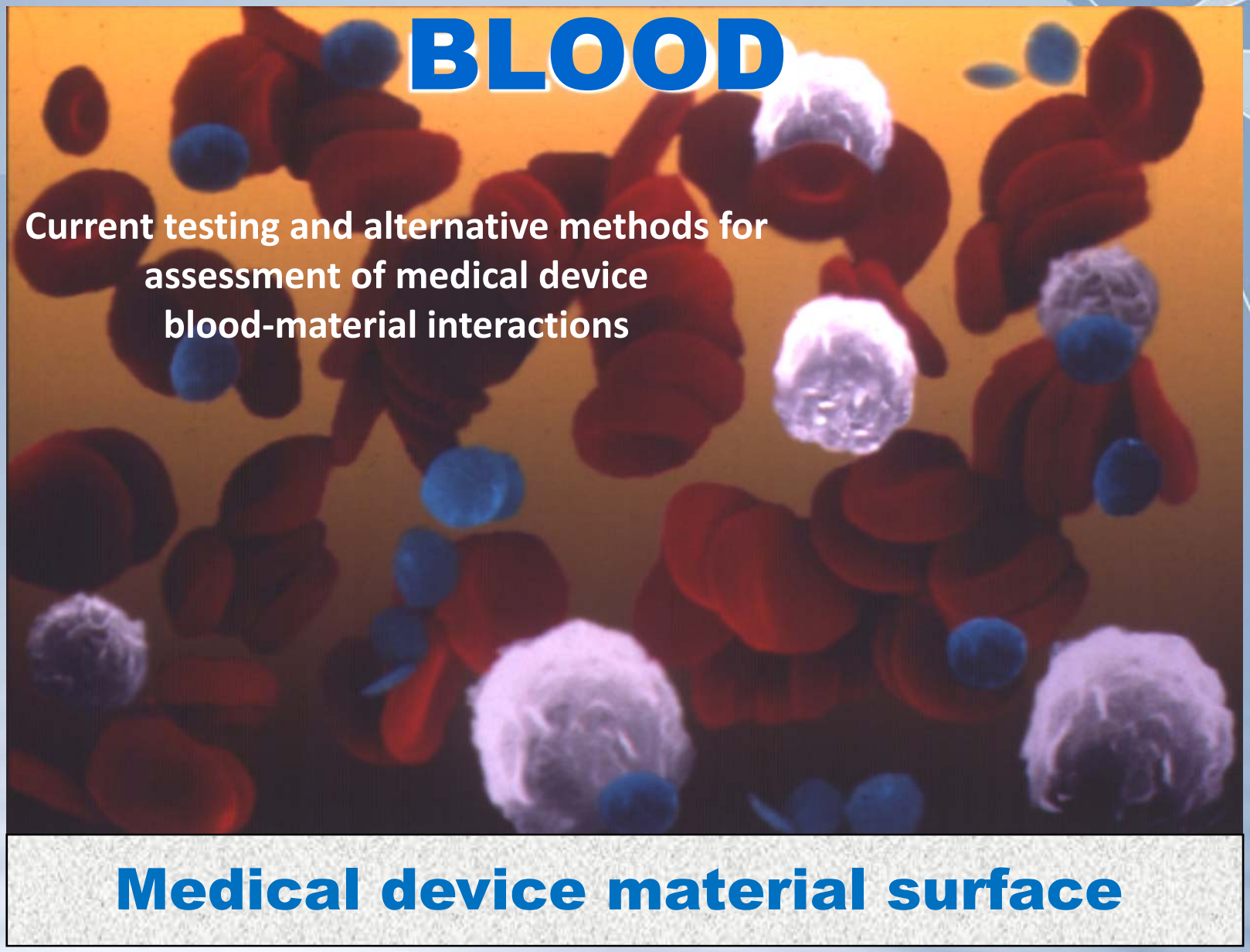
- other factors (patient, physician, etc..)



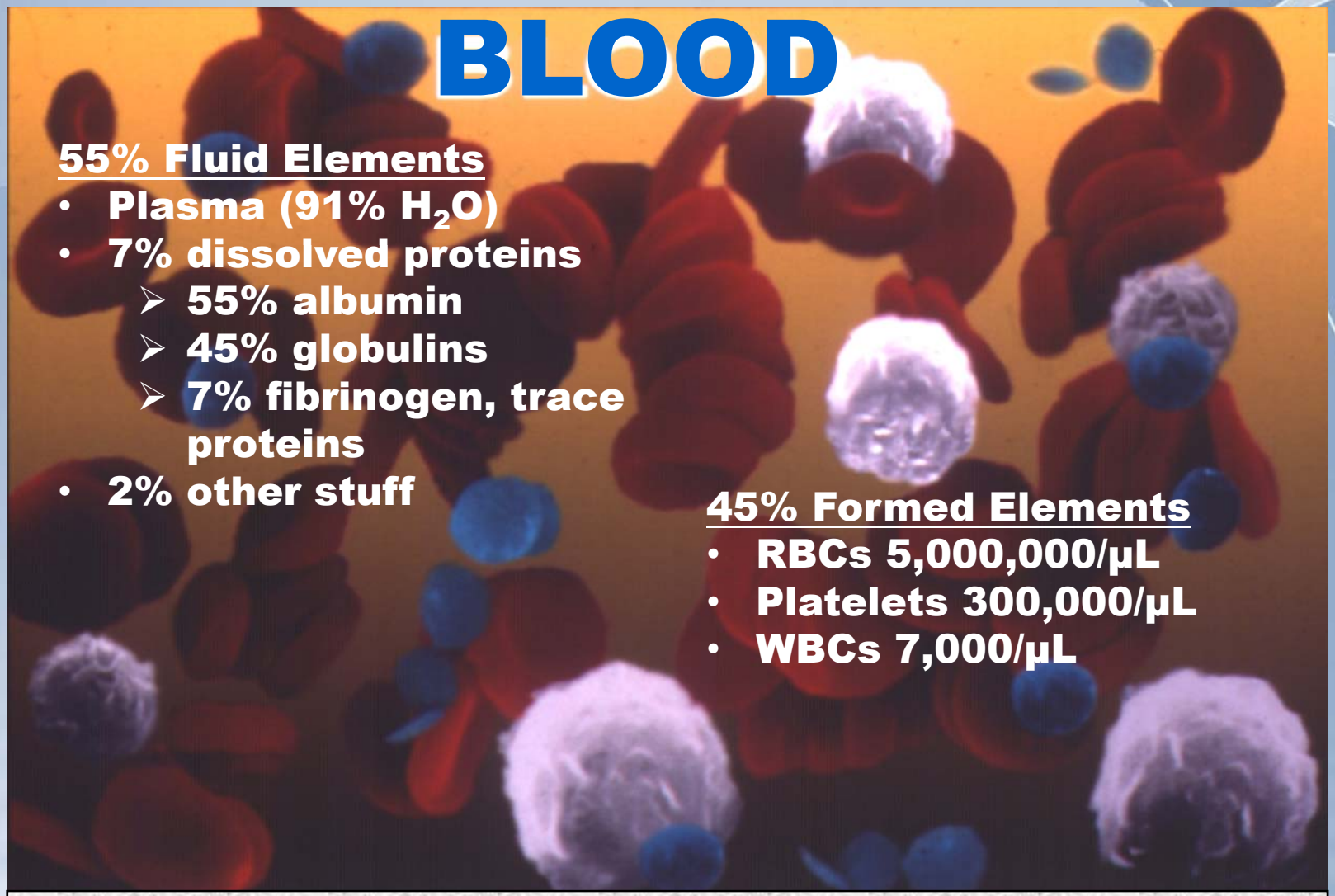







BLOOD
Current testing and alternative methods for assessment of medical device blood-material interactions
Medical device material surface

BLOOD



55% Fluid Elements

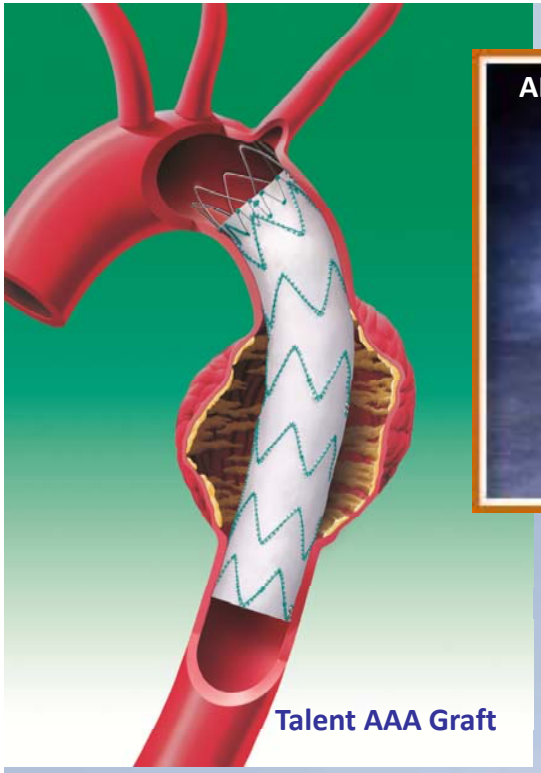
- Plasma (91% H_2O)
- 7% dissolved proteins
  - 55% albumin
  - 45% globulins
  - 7% fibrinogen, trace proteins
- 2% other stuff

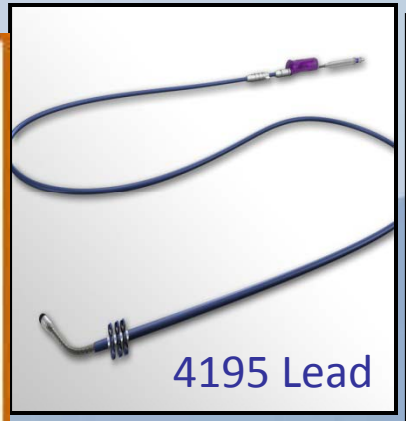
45% Formed Elements

- RBCs 5,000,000/µL
- Platelets 300,000/µL
- WBCs 7,000/µL

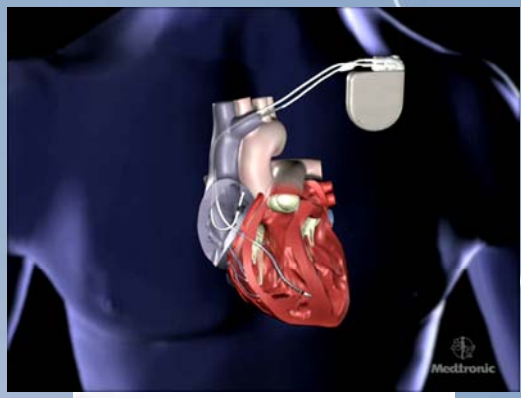
Medical device material surface

Many Products w. Blood Interface


Talent AAA Graft

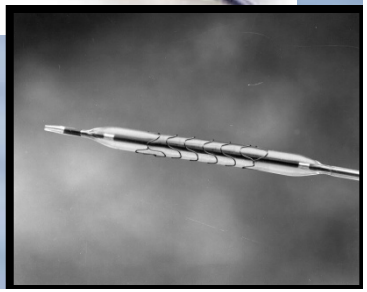

4195 Lead


Open Pivot Valve




Core Valve


Endeavor Stent



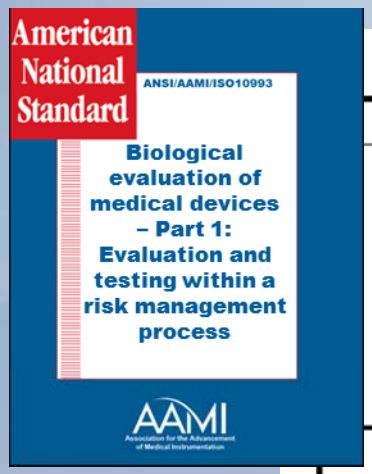


Table A.1 – Evaluation tests for consideration (ISO10993-Part 1)

Medical device categorization by			Biological effect							
Nature of body contact (see 5.2)		Contact duration (see 5.3) A — Limited (≤ 24 h) B — prolonged (>24 h to 30 days) C — permanent (> 30 days)	Cytotoxicity	Sensitization	Irritation or intracutaneous	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
Surface device	Skin	A	X[a]	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
External communicating device	Blood path, indirect	A	X	X	X	X				X
		B	X	X	X	X				X
		C	X	X		X	X	X		X
	Tissue/bone/dentin	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Circulating blood	A	X	X	X	X				X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X
Implant device	Tissue/bone	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Blood	A	X	X	X	X	X		X	X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X

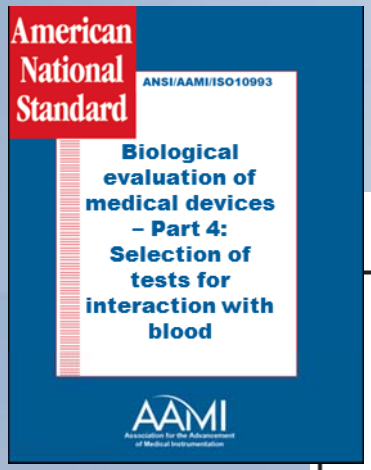


Table 1 — Circulating blood contacting devices or device components and the categories of appropriate testing — External communicating devices

Device examples	Test category				
	Thrombosis	Coagulation	Platelets	Hematology	Complement system
Catheters in place for less than 24 h (e.g. atherectomy devices, guide wires)	x[a]			x[b]	
Blood monitors	x[a]			x[b]	
Blood storage and administration equipment, blood collection devices, extension sets		x	x	x[b]	x[c]
Catheters in place for more than 24 h (e.g., intravascular endoscopes, intravascular ultrasound, lasers systems, retrograde coronary perfusion catheters)	x[a]			x[b]	x
Cell savers		x	x	x[b]	
Devices for absorption of specific substances from blood		x	x	x	x
Donor and therapeutic aphaeresis equipment and cell separation systems		x	x	x	x
Extracorporeal membrane oxygenators system Hemodialysis/hemofiltration equipment Percutaneous circulatory support devices	x[a]			x	x
Leukocyte removal filter		x	x	x[b]	x

[a] As stated in 3.8, thrombosis is an *in-vivo* or *ex-vivo* phenomenon. It is recognized that coagulation and platelet responses are involved in this process. Therefore, it is up to the manufacturer to decide if specific testing in the coagulation and platelet test categories is appropriate for their device.

[b] Hemolysis testing only.

[c] Only for aphaeresis equipment and related procedures.

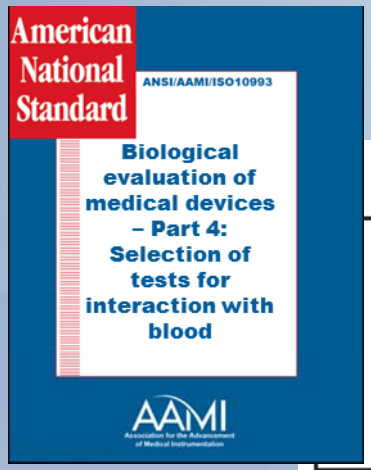


Table 2 — Circulating blood contacting devices or device components and the categories of appropriate testing — Implant devices

Device examples	Test category				
	Thrombosis	Coagulation	Platelets	Hematology	Complement system
Annuloplasty rings, mechanical heart valves	x[a]			x[b]	
intra-aortic balloon pumps	x[a]			x	
total artificial hearts, ventricular-assist devices	x[a]			x	x
Embolization devices	x[a]			x[b]	
Endovascular grafts	x[a]			x[b]	x
Implantable defibrillators and cardioverters	x[a]			x[b]	
Pacemaker leads	x[a]			x[b]	
Prosthetic (synthetic) vascular grafts and patches, including arteriovenous shunts	x[a]			x[b]	x
Stents	x[a]			x[b]	
Tissue heart valves	x[a]			x[b]	
Tissue vascular grafts and patches, including arteriovenous shunts	x[a]			x[b]	
Vena cava filters	x[a]			x[b]	

[a] As stated in 3.8, thrombosis is an *in-vivo* or *ex-vivo* phenomenon. It is recognized that coagulation and platelet responses are involved in this process. Therefore, it is up to the manufacturer to decide if specific testing in the coagulation and platelet test categories is appropriate for their device.

[b] Hemolysis testing only.

Table 3 — Test methods for external communicating devices

Test Category	Method
Thrombosis	Percent occlusion
	Flow reduction
	Gravimetric analysis (thrombus mass)
	Light microscopy (adhered platelets, leukocytes, aggregates, erythrocytes, fibrin, etc.)
	Pressure drop across device
	Labelled antibodies to thrombotic components
	Scanning EM (platelet adhesion and aggregation; platelet and leukocyte morphology, fibrin)
Coagulation	PTT (non-activated)
	Thrombin Generation - Specific coagulation factor assays; FPA, D-dimer, F_{1+2}, TAT
Platelets	Platelet count / adhesion
	Platelet aggregation
	Template bleeding time
	Platelet function analysis
	PF-4,β-TG ; thromboxane B2
	Platelet activation markers,
	Platelet microparticles
	Gamma imaging of radio labelled platelets, ^{111}In - labelled platelet survival
Haematology	Leukocyte count with or without differential
	Leukocyte activation
	Haemolysis
	Reticulocyte count; activation-specific release products of peripheral blood cells (i.e. granulocytes)
Complement system	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9, CH50, C3 convertase, C5 convertase

Table 4 — Test methods for implant devices

Test category	Evaluation Method
Thrombosis	Scanning EM (platelet adhesion and aggregation; platelet and leukocyte morphology; fibrin)
	Percent occlusion
	Flow reduction
	Labelled antibodies to thrombotic components
	Autopsy of devices (gross and microscopic) ; histopathology
	Autopsy of distal organs (gross and microscopic) : histopathology
Coagulation	Specific coagulation factor assay; FPA, D-dimer, F_{1+2}, PAC-1, S-12, TAT
	PTT(non-activated), PT, TT; Plasma fibrinogen; FDP
Platelets	PF-4, β-TG, thromboxane B2,
	Platelet activation markers
	Platelet microparticles
	Gamma imaging of radio labelled platelets ^{111}-In labelled platelet survival
	Platelet function analysis
	Platelet count / adhesion
	Platelet aggregation
Haematology	Leukocyte count with or without differential;
	Leukocyte activation
	Haemolysis
	Reticulocyte count; activation specific release products of peripheral blood cells (i.e. , granulocytes)
Complement system	C3a, C5a, TCC, Bb, iC3b, C4b, SC5b-9, CH 50, C3 convertase, C5 convertase

Test Category	Table 3. External Communicating Devices	Table 4. Implant Devices
Thrombosis	Percent occlusion	Percent occlusion
	Flow reduction	Flow reduction
	Gravimetric analysis (thrombus mass)	Gravimetric analysis (thrombus mass) (mfw added)
	Labeled antibodies to thrombotic components	Labeled antibodies to thrombotic components
	Light microscopy (adhered platelets, leucocytes, aggregates, erythrocytes, fibrin, etc.)	Light microscopy (adhered platelets, leucocytes, aggregates, erythrocytes, fibrin, etc.) (mfw added)
	Scanning E.M. (platelet adhesion and aggregation; platelet and leucocyte morphology; fibrin)	Scanning E.M. (platelet adhesion and aggregation; platelet and leucocyte morphology; fibrin)
	Pressure drop across device	Autopsy of devices (gross and microscopic) histopathology
		Autopsy of distal organs (gross and microscopic); histopathology
Coagulation	PTT (non-activated)	PTT (non-activated), PT, TT; plasma fibrinogen; FDP
	Thrombin generation – coagulation assays; FPA, D-dimer, F_{1+2}, TAT	Specific coagulation factor assay; FPA, D-dimer, F_{1+2}, PAC-1, S-12, TAT
Platelets	Platelet count/adhesion	Platelet count/adhesion
	Platelet aggregation	Platelet aggregation
	Platelet microparticles	Platelet microparticles
	Platelet function analysis	Platelet function analysis
	Platelet activation markers	Platelet activation markers
	PF-4, β-TG, thromboxane B2	PF-4, β-TG, thromboxane B2
	Gamma imaging of radiolabeled platelets, ^{111}In-labeled platelet survival	Gamma imaging of radiolabeled platelets, ^{111}In-labeled platelet survival
	Template bleeding time	^{111}In-labeling is recommended for prolonged or repeated use (>24 hours to 30 days) and permanent contact (>30 days)
Hematology	Leucocyte count with or w/o differential	Leucocyte count with or w/o differential
	Leucocyte activation	Leucocyte activation
	Hemolysis	Hemolysis
	Reticulocyte count, activation-specific release products of peripheral blood cells (i.e., granulocytes)	Reticulocyte count, activation-specific release products of peripheral blood cells (i.e., granulocytes)
Complement	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9, CH50, C3 and/or convertase	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9, CH50, C3 and/or C5 convertase,

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Hematology	Leucocyte count with or w/o differential	Leucocyte count with or w/o differential
	Leucocyte activation	Leucocyte activation
	Hemolysis	Hemolysis
	Reticulocyte count, activation-specific release products of peripheral blood cells (i.e., granulocytes)	Reticulocyte count, activation-specific release products of peripheral blood cells (i.e., granulocytes)
Complement	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9, CH50, C3 and/or convertase	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9, CH50, C3 and/or C5 convertase

‘Short list’ of tests used to assess interaction with blood

Test Category	Tests
Thrombosis	**Gross analysis, light microscopy, SEM** (in vivo implant study) **NAVI/AVI model** [1]
Coagulation	Thrombin measurements (TAT, F1.2) [2] ; fibrin measurement (FPA) [2]
Platelets	Platelet counts, platelet granule release products (βTG and PF4) [2]
Hematology	**Hemolysis**, basic blood counts (Coulter analysis), white blood cell activation (PMN elastase release) [2]
Complement System	**C3a** , C5a, **SC5b9** [3]

1 not standardized, FDA only
2 under-utilized (alternative)
3 over-utilized, not standardized
red bold = frequently used

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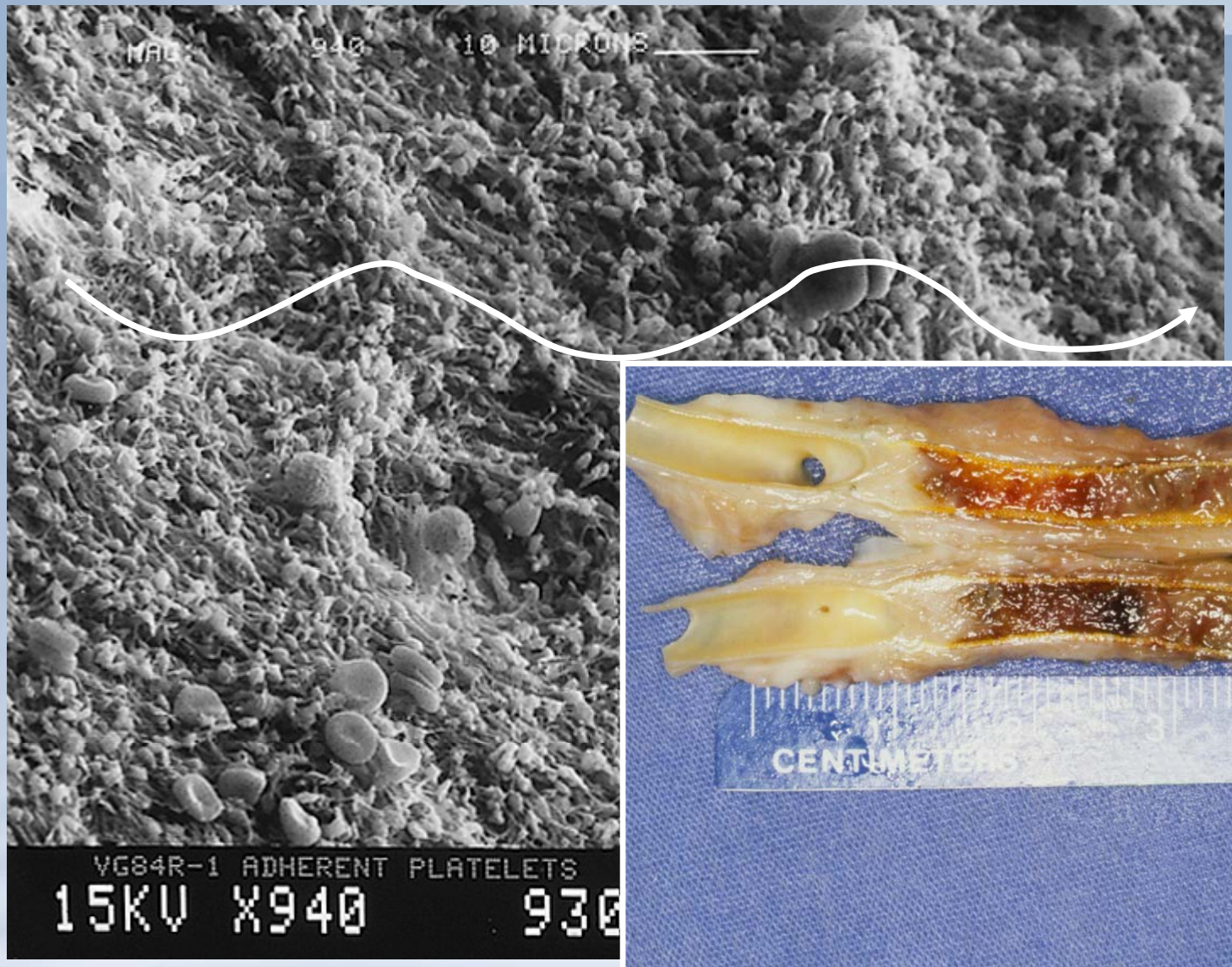
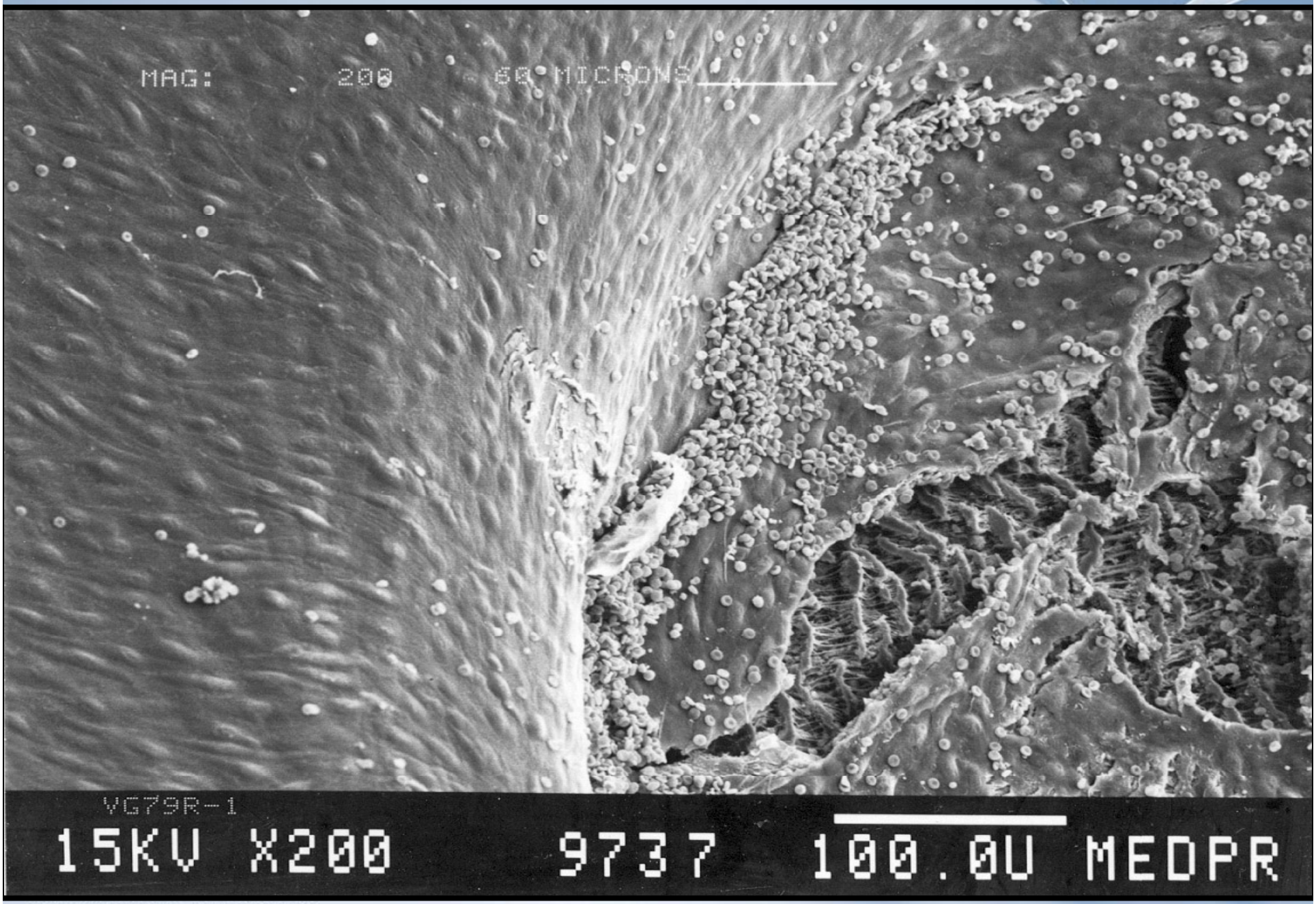
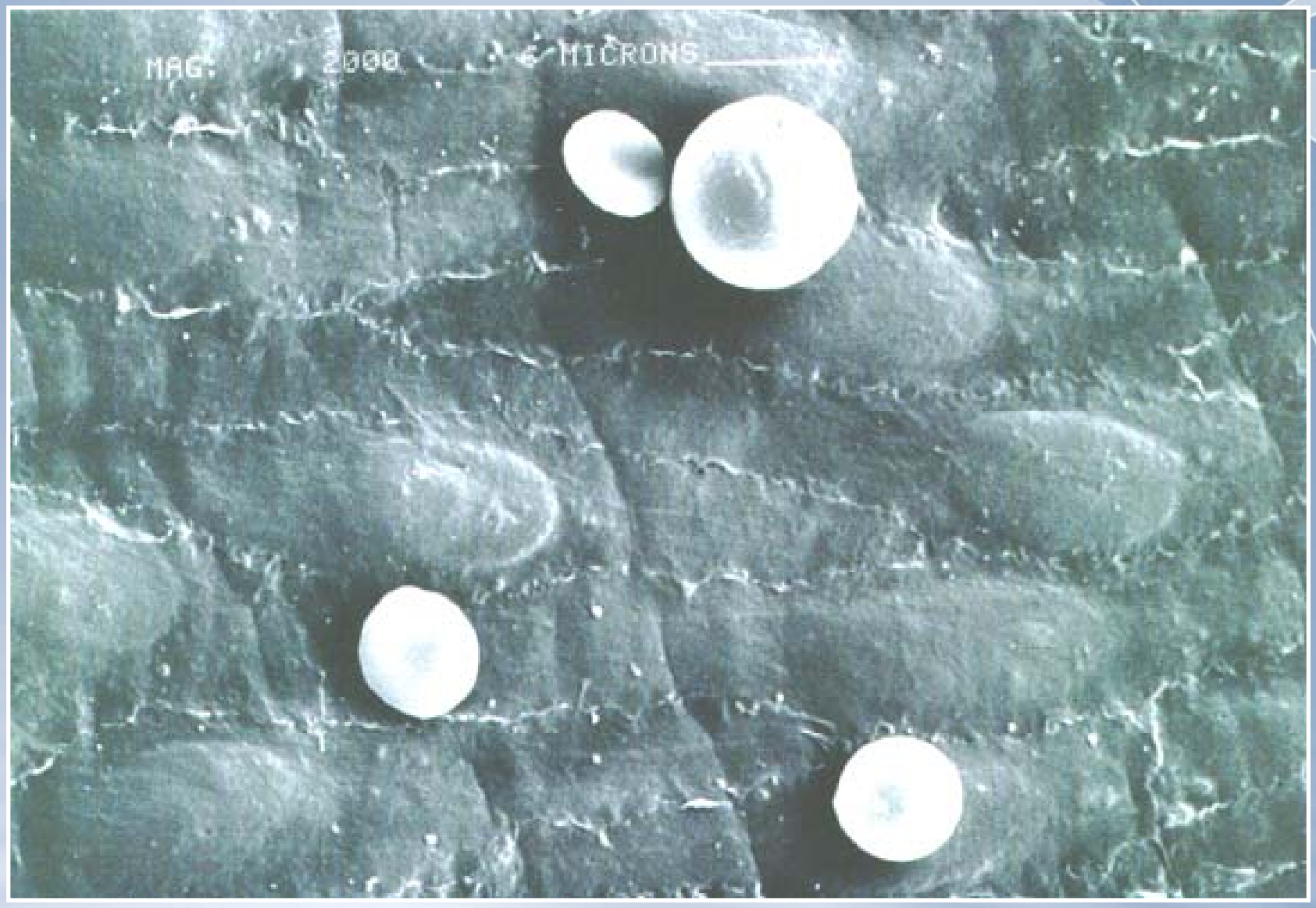
Thrombosis: device = vascular graft (SEM and gross analysis)



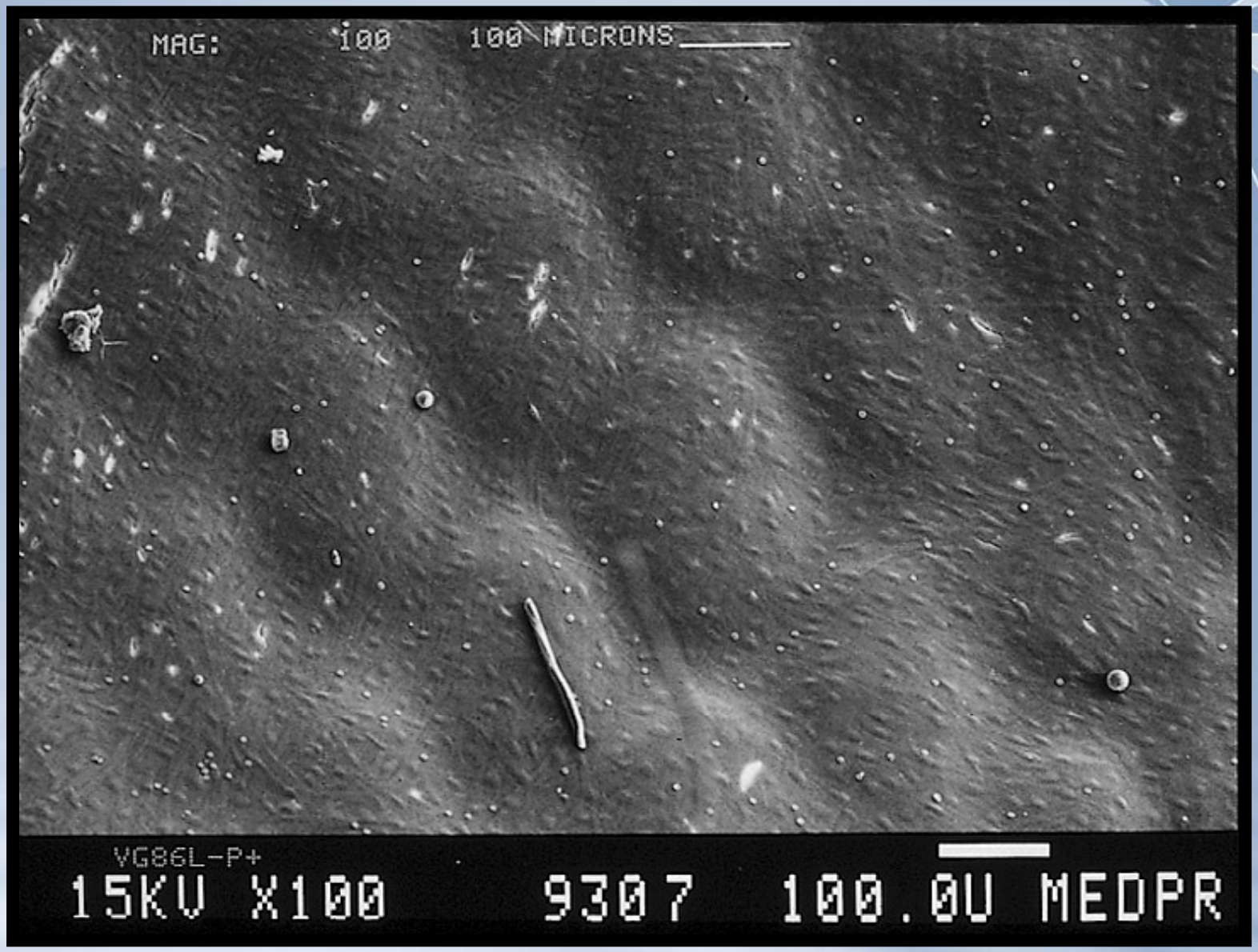
Thrombosis: device = vascular graft (SEM and light microscopy)



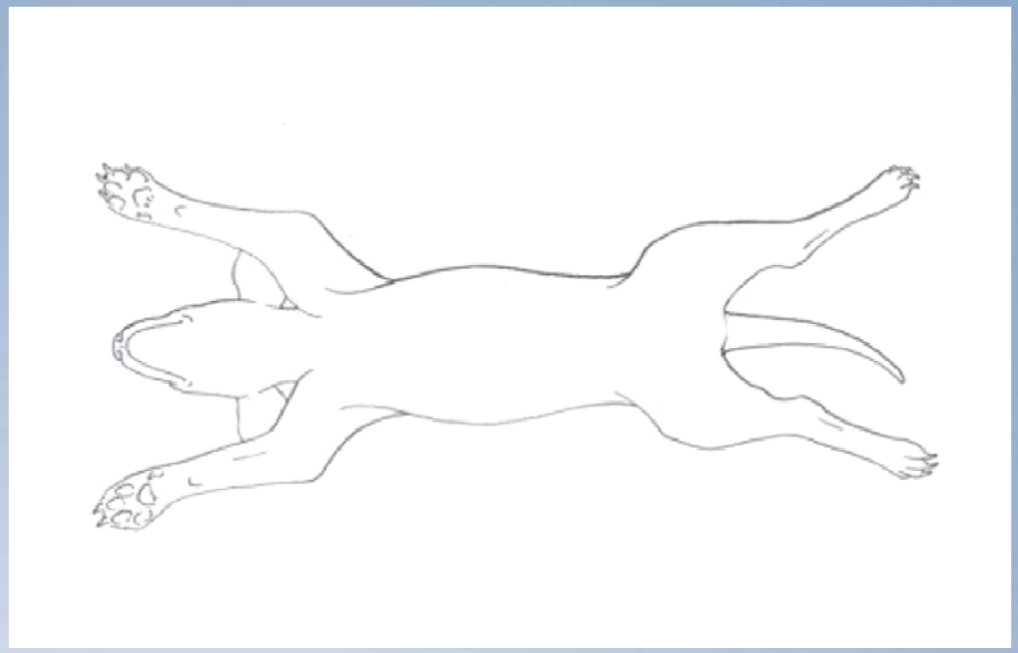
Thrombosis: device = vascular graft (SEM for endothelialization)



Thrombosis: device = vascular graft (SEM for endothelialization)

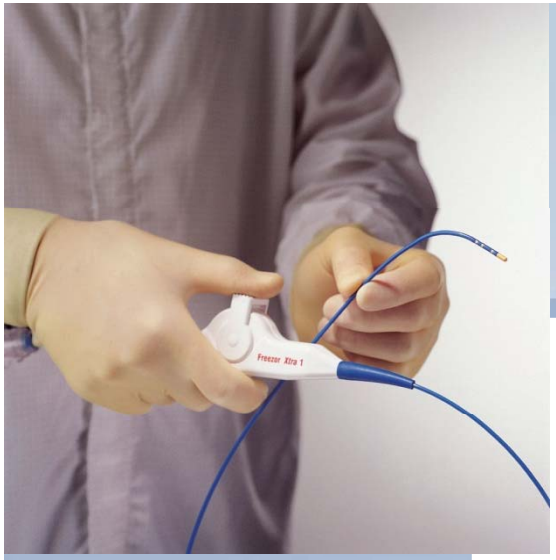


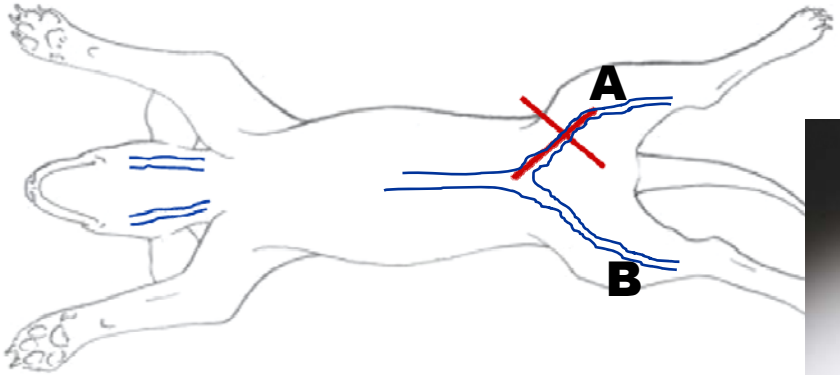
The NAVI Model*



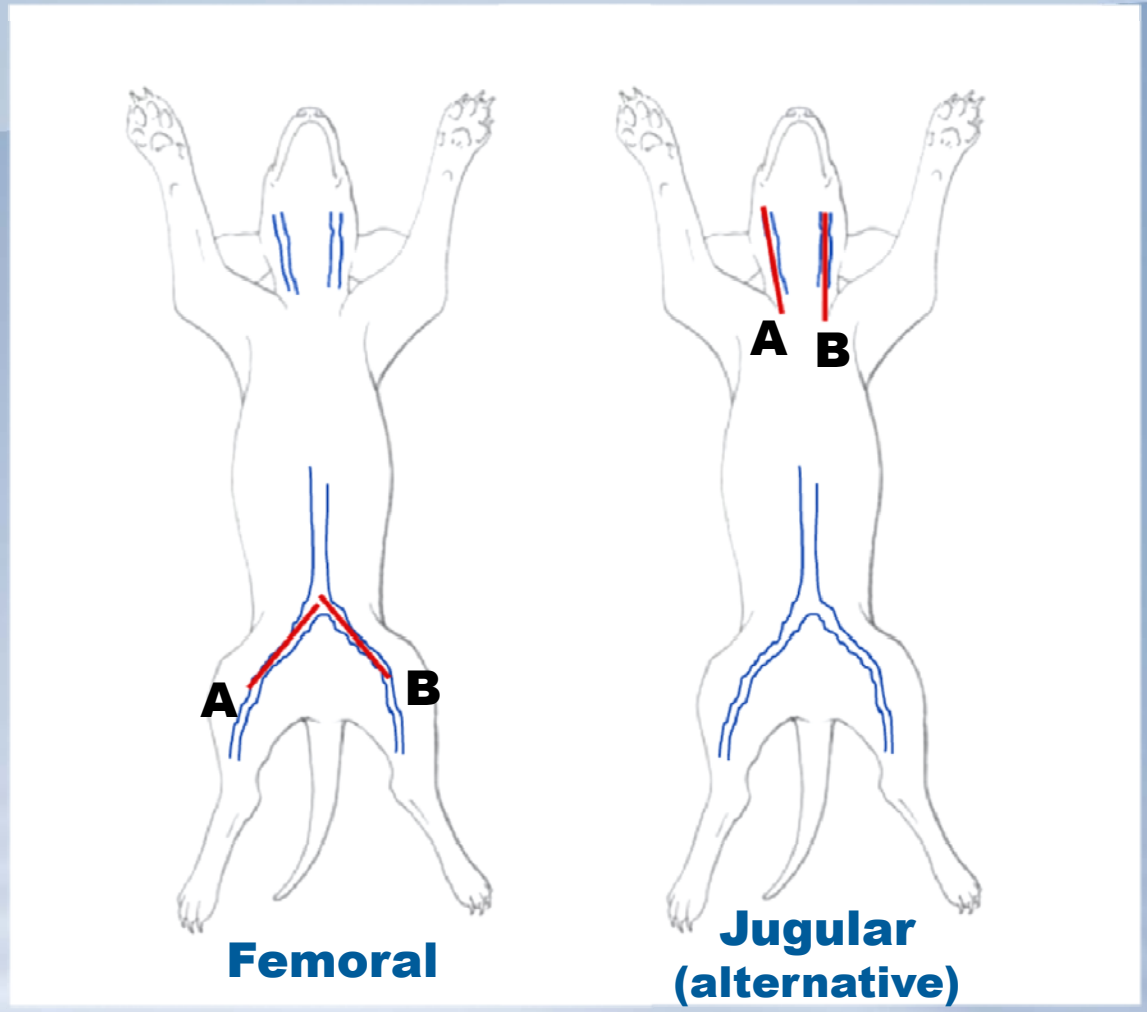
Canine, Porcine, Ovine

*non-anticoagulated venous implant model

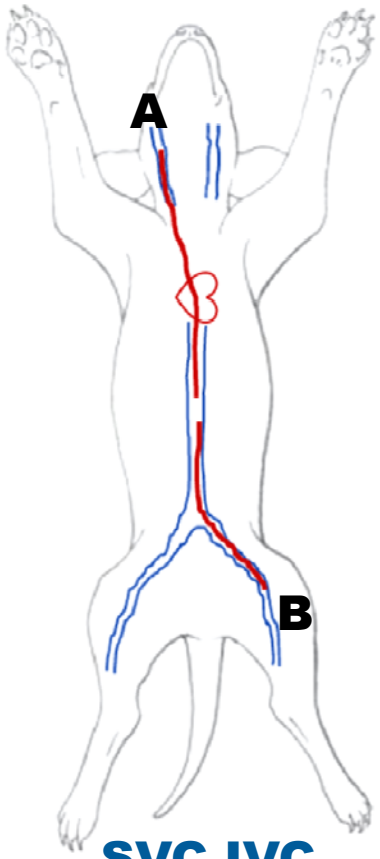




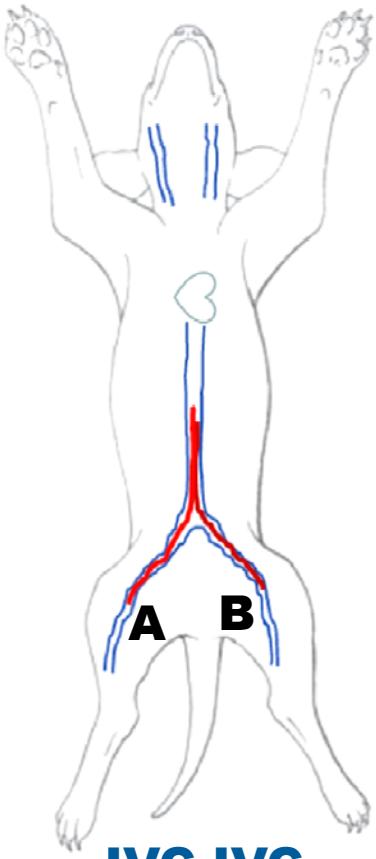
Canine Femoral Vein (most common)


A
B
Femoral
A
B
Jugular
(alternative)

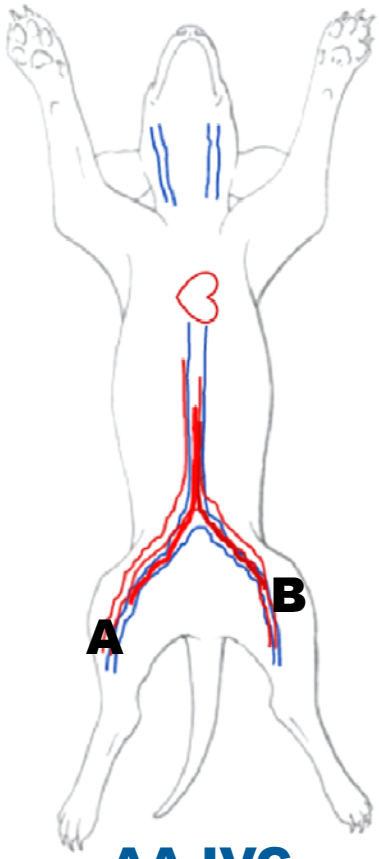
Other Implant Variations (less common)



SVC-IVC



IVC-IVC



AA-IVC

NAVI Model – Scoring

Score	Thrombus Formation Score Description (Lab A)
0	No significant thrombosis (very small clot acceptable at insertion)
1	Minimal thrombosis, one location.
2	Minimal thrombosis, multiple locations.
3	Significant thrombosis, ≤ ½ the length of the implant, vessel patent.
4	Significant thrombosis, > ½ the length of the implant, vessel patent.
5	Vessel completely occluded.

A test article that receives a score of 3 or greater is considered failing and does not meet the requirements of the protocol

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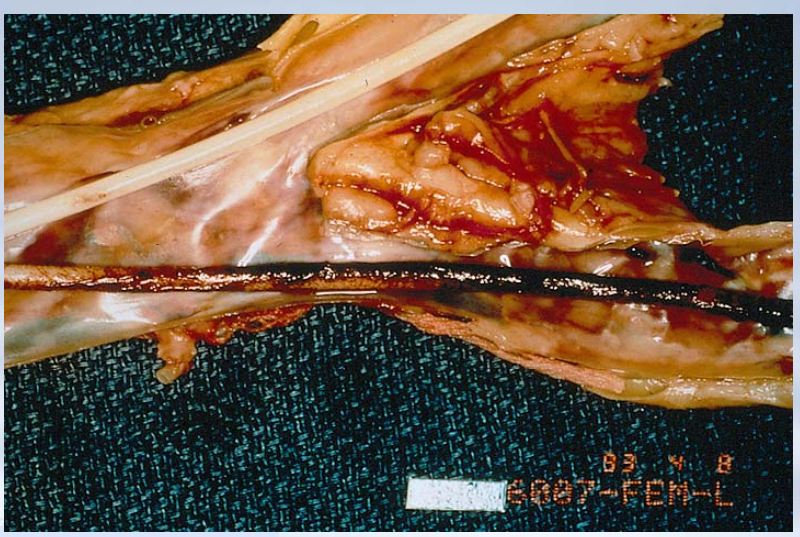

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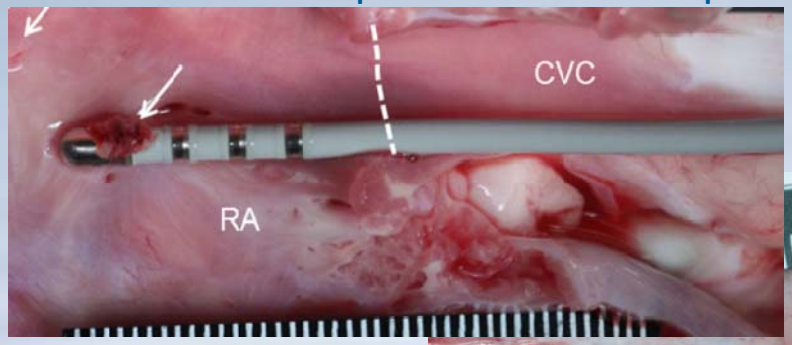

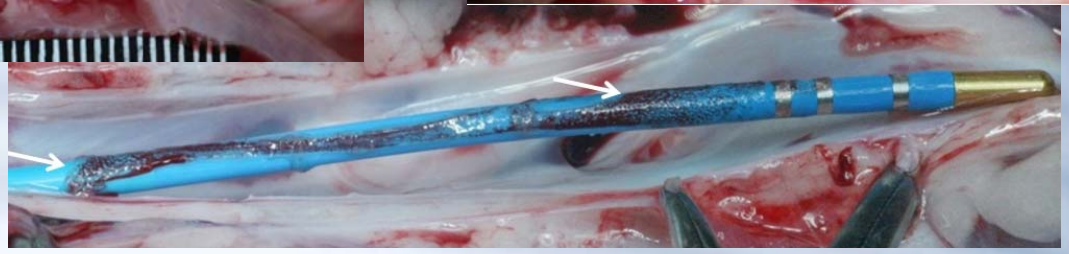

NAVI Model Caveats

- ✓The implant position (P)
- ✓The implant technique (IT)
- ✓The extent of device-vessel wall contact (tissue damage, TD)
- ✓Time/incubation period (IP)
- ✓The explant technique (ET)
- ✓The material/material surface (M)
- ✓Nonthromboadherent materials get labeled nonthrombogenic (thrombogenic-non-thromboadherent, TNT)
- ✓The recipient/subject thrombotic potential (STP)
- ✓Statistical power (SP)
- ✓Evaluator expertise (EE)

NAVI Score = f (P, IT, TD, IP, ET, M, TNT, STP, SP, EE)

WANT:

NAVI Score = f (P, IT, TD, IP, ET, **M**, TNT, STP, SP, EE)

NAVI Model Caveats

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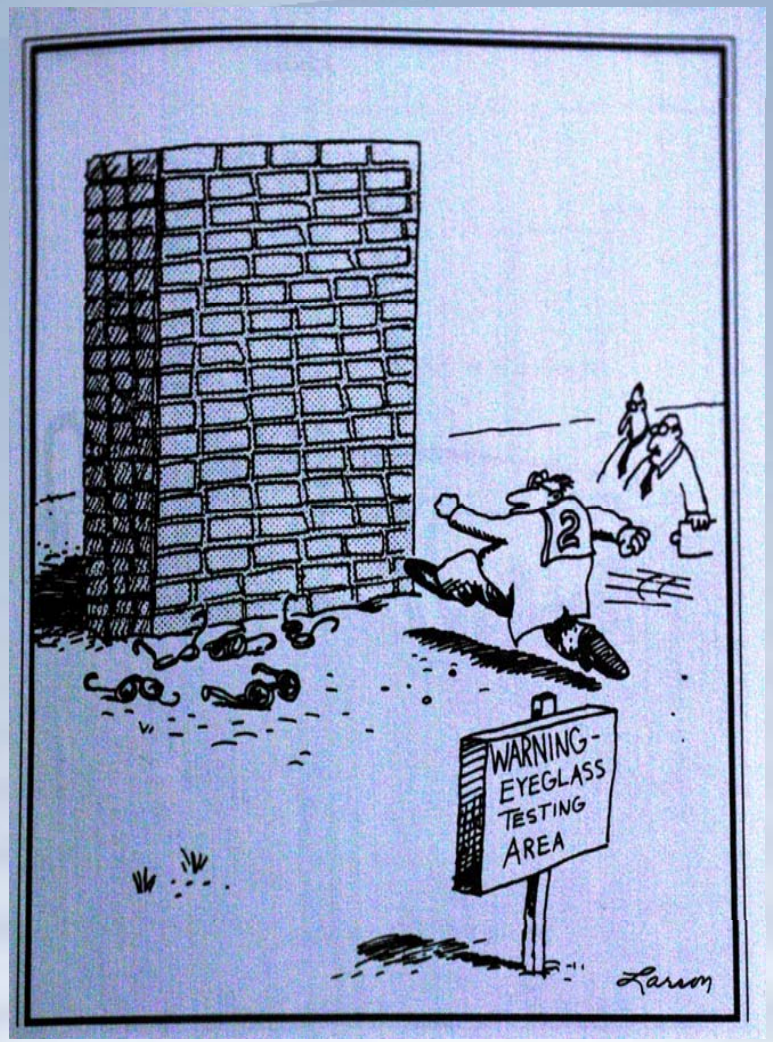
NAVI Score = f (P, IT, TD, IP, ET, M, TNT, STP, SP, EE)

REALITY:

NAVI Score = f (**P**, IT, **TD**, IP, ET, **M**, TNT, STP, SP, **EE**)

NAVI Score = f (P, IT, TD, IP, ET, M, TNT, STP, SP, EE)



- ✓ Coagulation, thrombosis, fibrinolysis, are natural and complex processes
- ✓ Measuring clinically-significant thrombosis on medical devices requires more validated methods

‘Short list’ of tests used to assess interaction with blood

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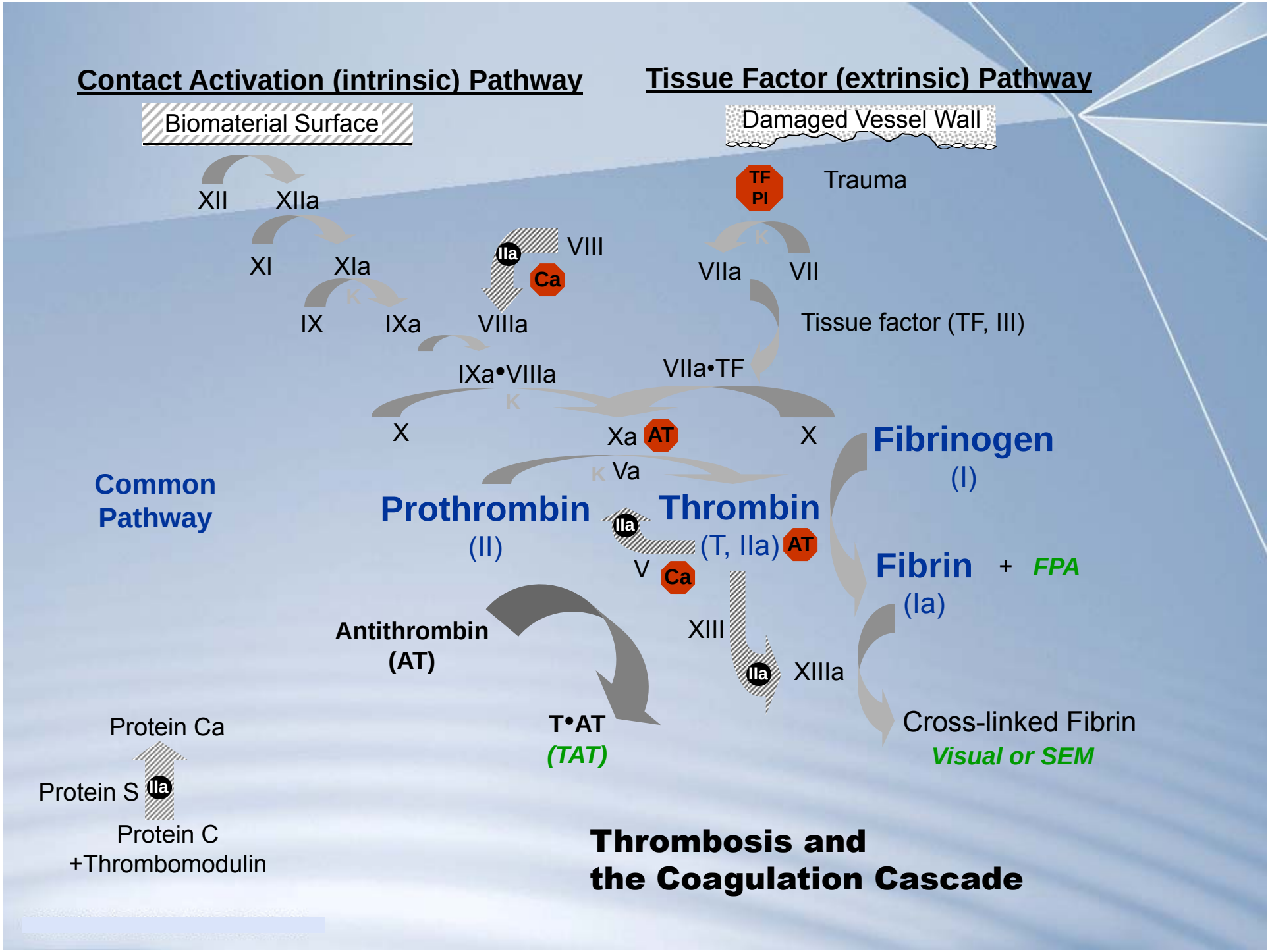
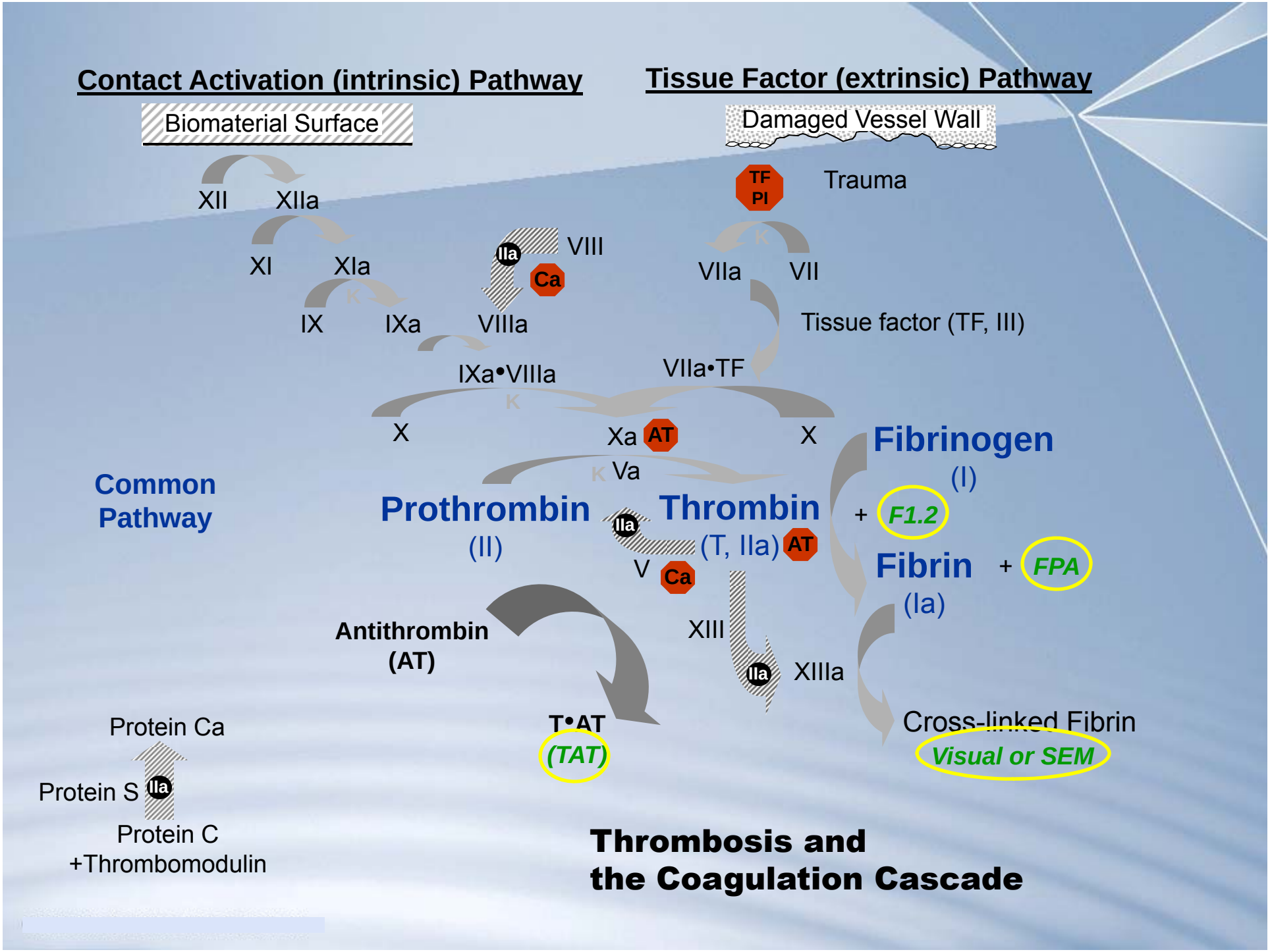
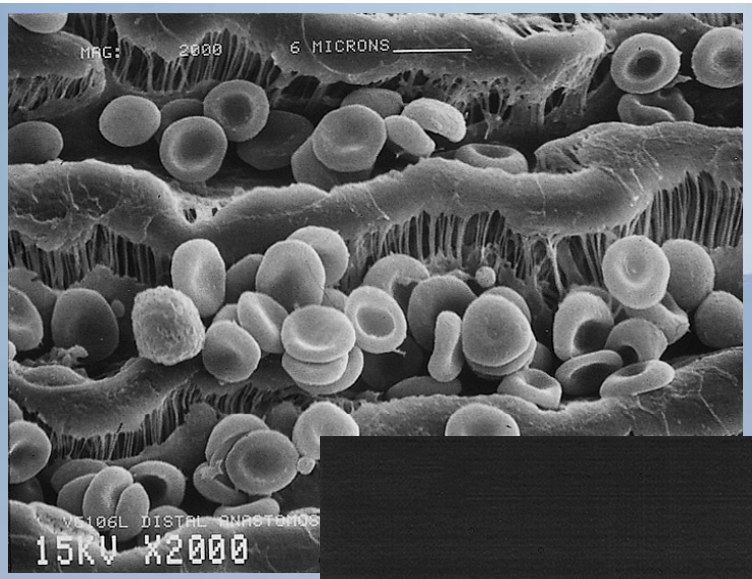
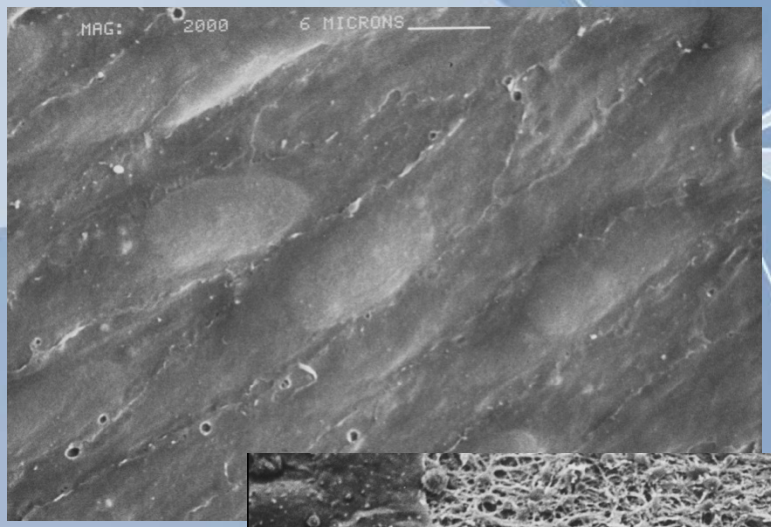
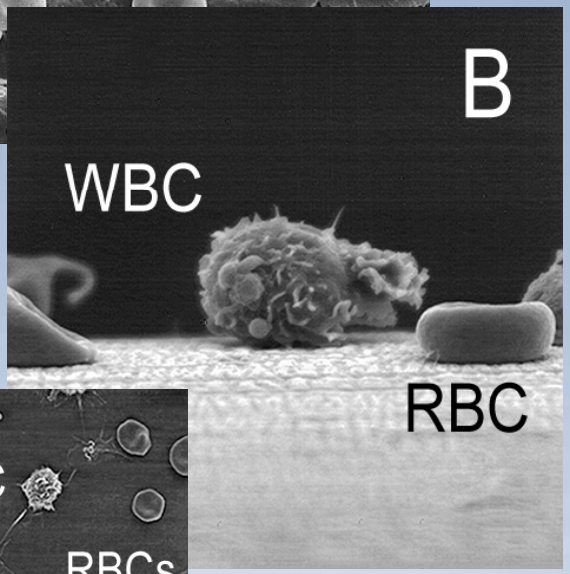
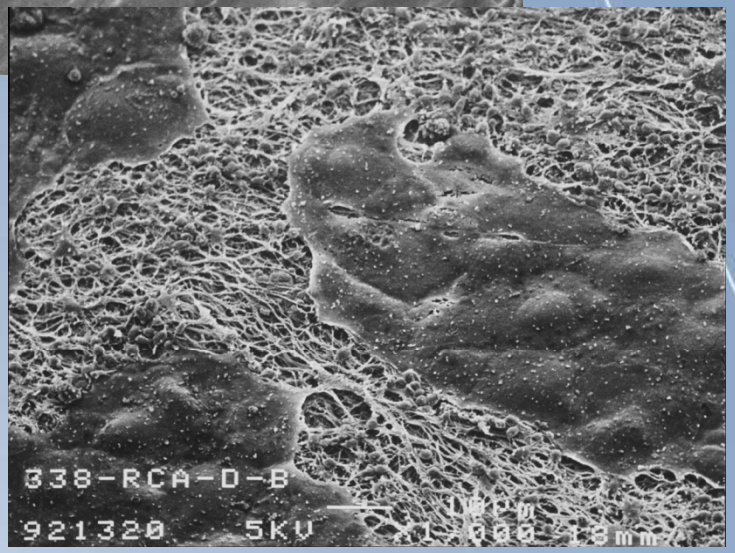

Contact Activation (intrinsic) Pathway
Biomaterial Surface
XII
XIIa
XI
XIa
IX
IXa
VIII
IIa
Ca
VIIIa
IXa•VIIIa
X
Tissue Factor (extrinsic) Pathway
Damaged Vessel Wall
TF
PI
Trauma
VIIa
VII
Tissue factor (TF, III)
VIIa•TF
X
Common Pathway
Xa
AT
Va
Prothrombin
(II)
Thrombin
(T, IIa)
AT
V
Ca
Fibrinogen
(I)
Fibrin
(Ia)
+ FPA
XIII
XIIIa
Antithrombin
(AT)
T•AT
(TAT)
Cross-linked Fibrin
Visual or SEM
Protein Ca
Protein S
Protein C
+Thrombomodulin
Thrombosis and
the Coagulation Cascade

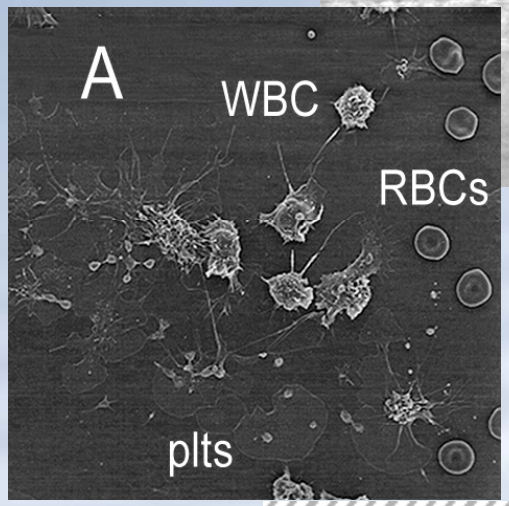

Contact Activation (intrinsic) Pathway
Biomaterial Surface
XII
XIIa
XI
XIa
IX
IXa
VIII
IIa
Ca
VIIIa
IXa•VIIIa
X
Tissue Factor (extrinsic) Pathway
Damaged Vessel Wall
TF
PI
Trauma
VIIa
VII
Tissue factor (TF, III)
VIIa•TF
X
Common Pathway
Xa
AT
Va
Prothrombin
(II)
Thrombin
(T, IIa)
AT
V
Ca
Fibrinogen
(I)
+
F1.2
Fibrin
(Ia)
+
FPA
XIII
XIIIa
Antithrombin
(AT)
T•AT
(TAT)
Cross-linked Fibrin
Visual or SEM
Protein Ca
Protein S
Protein C
+Thrombomodulin
Thrombosis and
the Coagulation Cascade







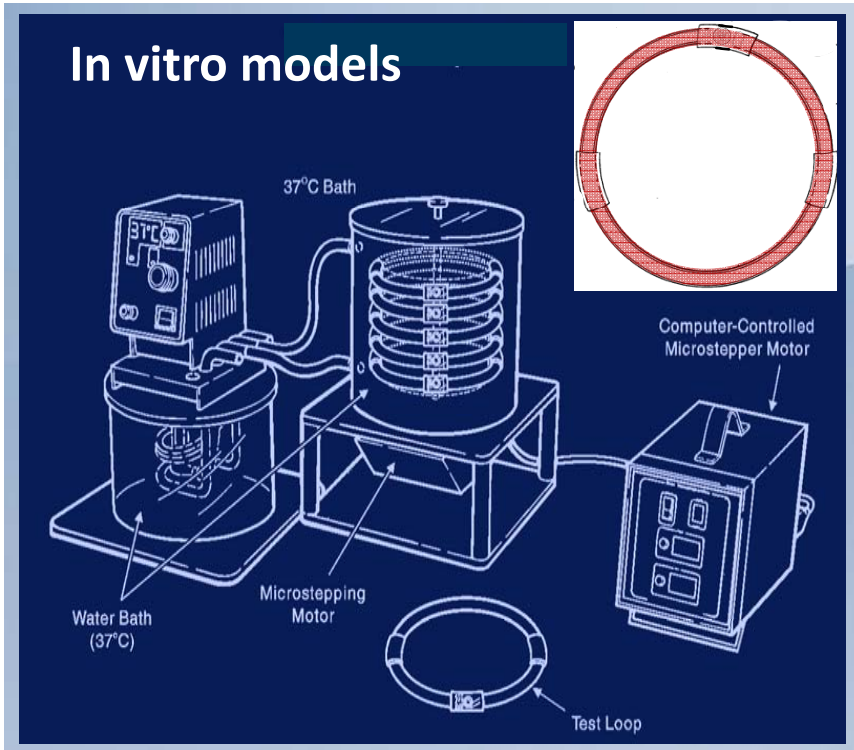




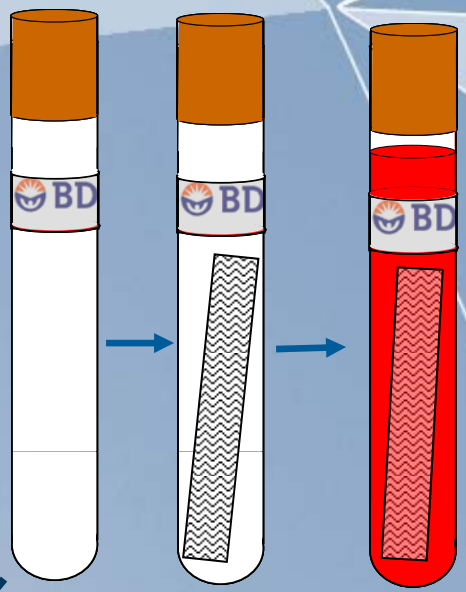
Damaged Vessel Wall

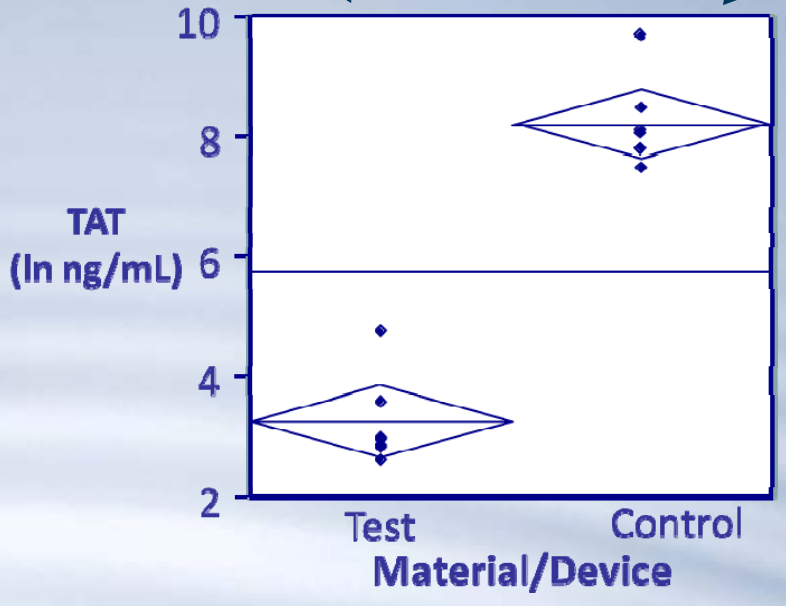
Biomaterial Surface

In vitro models

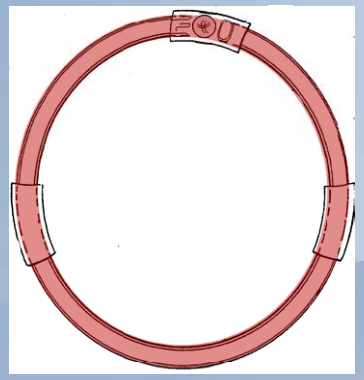


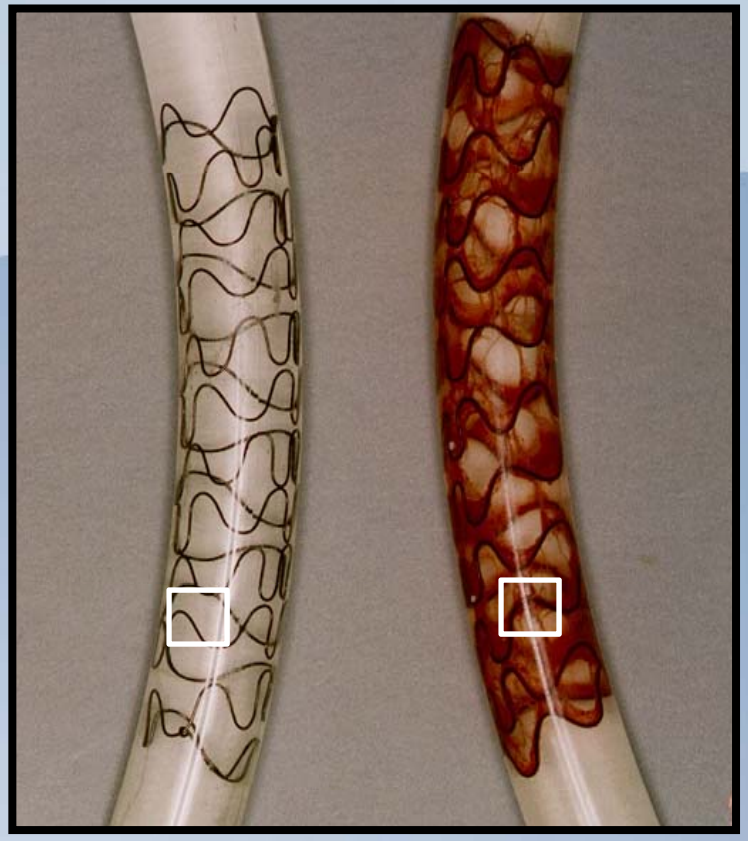
BD In vitro Vacutainer Model



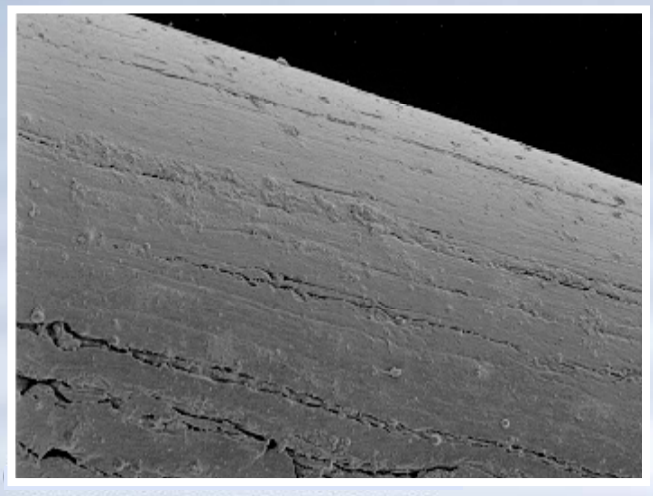


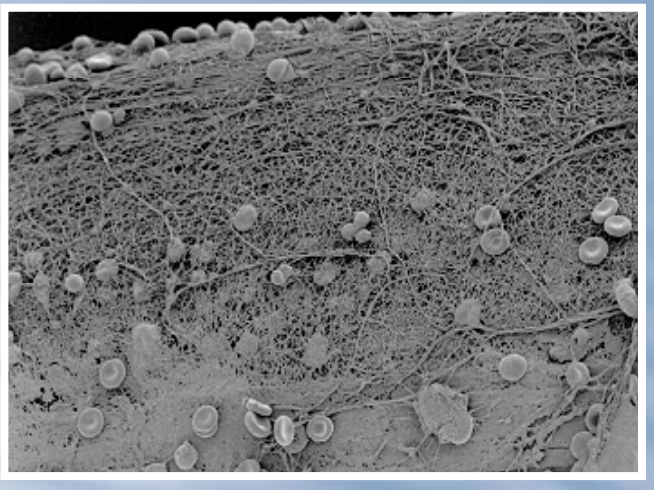
Caveat: test kits for human blood use only; animal models require antibody

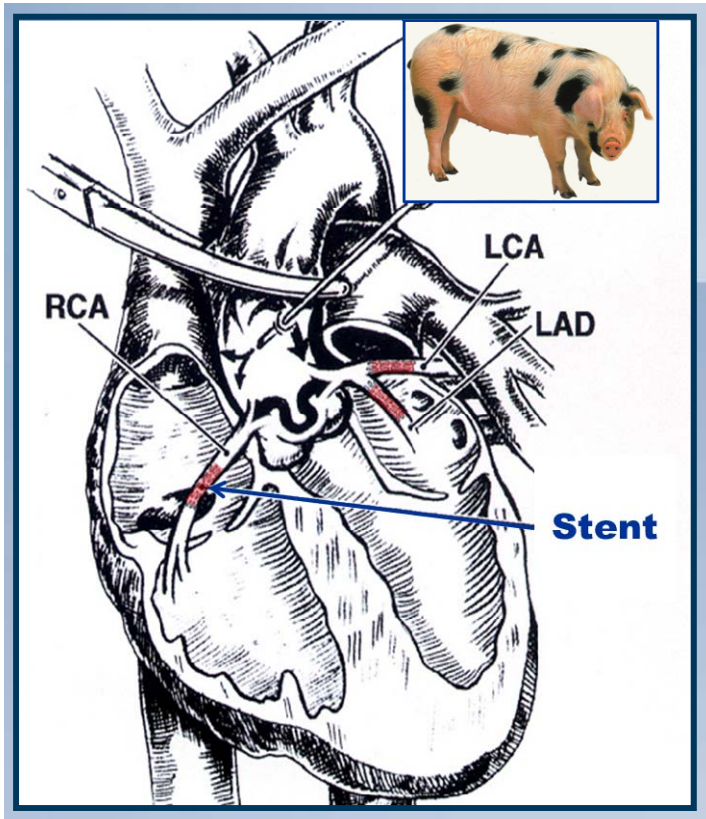


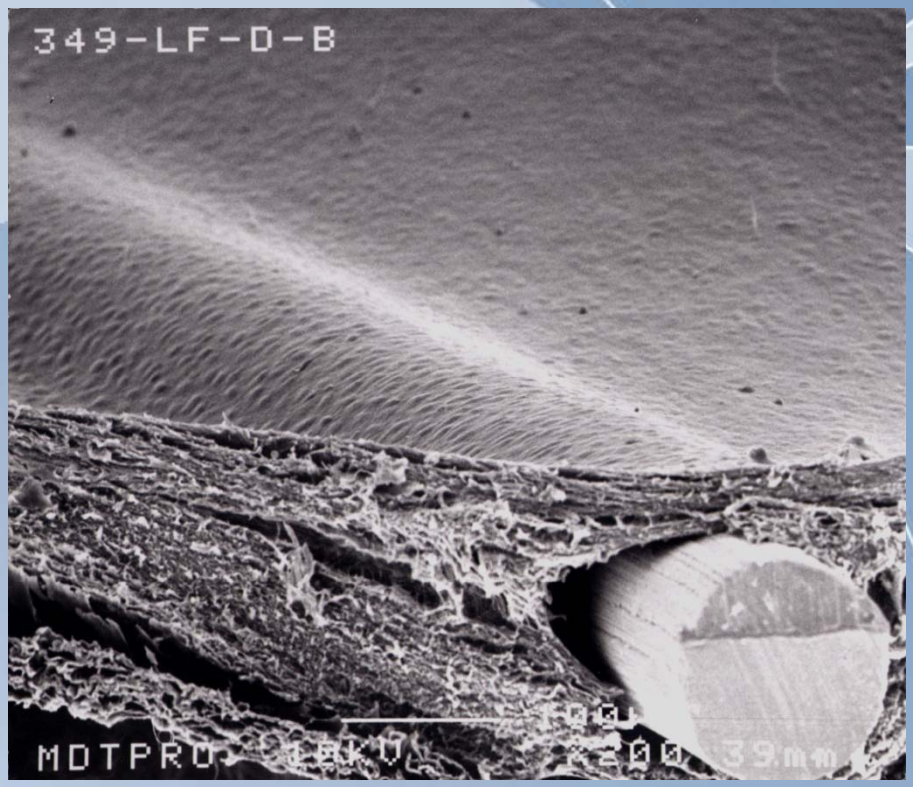


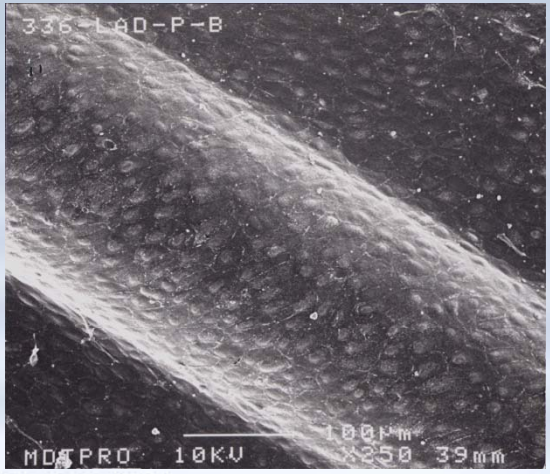
Test Control

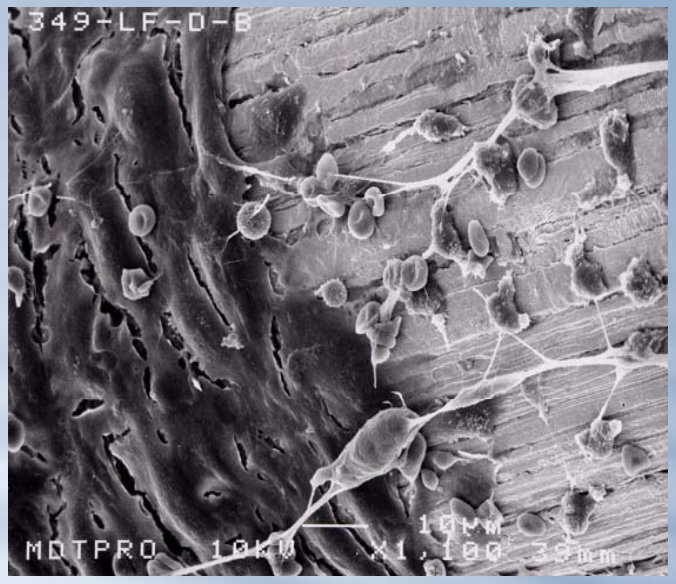





LCA
RCA
LAD
Stent


349-LF-D-B
MDTPRO


336-LAD-P-B
MDTPRO 10KV
X250 39mm


349-LF-D-B
MDTPRO 10KV
X1,100 39mm

‘Short list’ of tests used to assess interaction with blood

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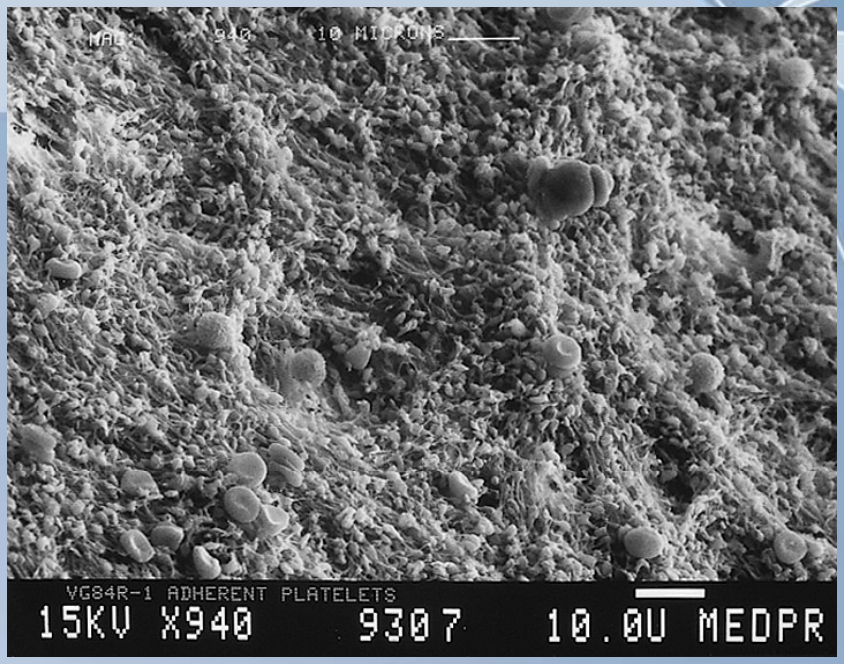
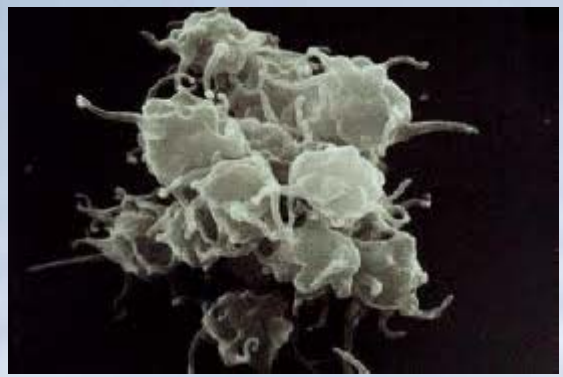
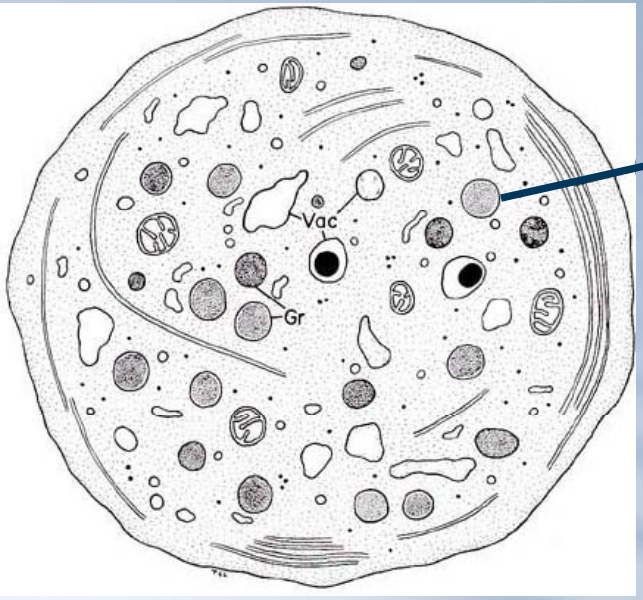
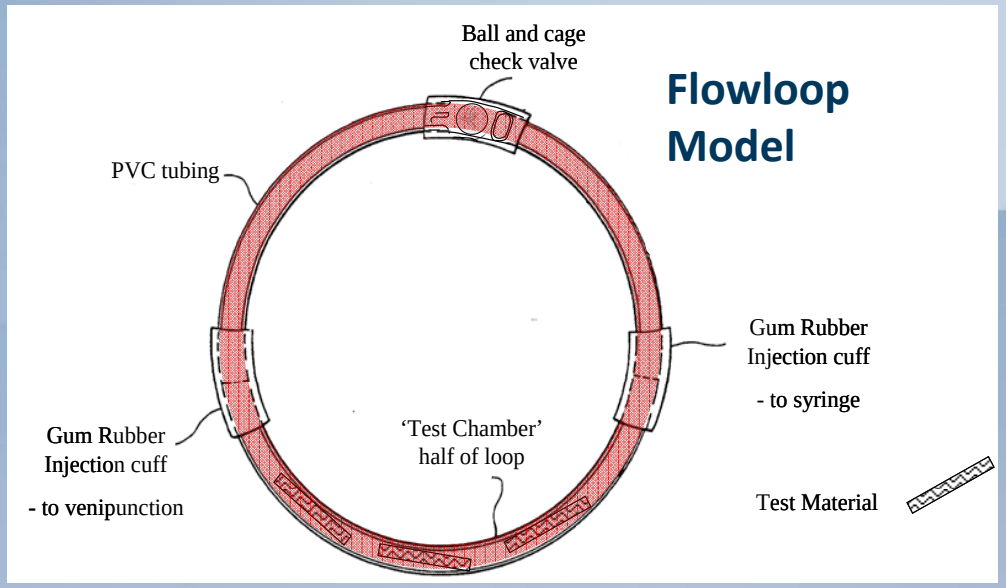
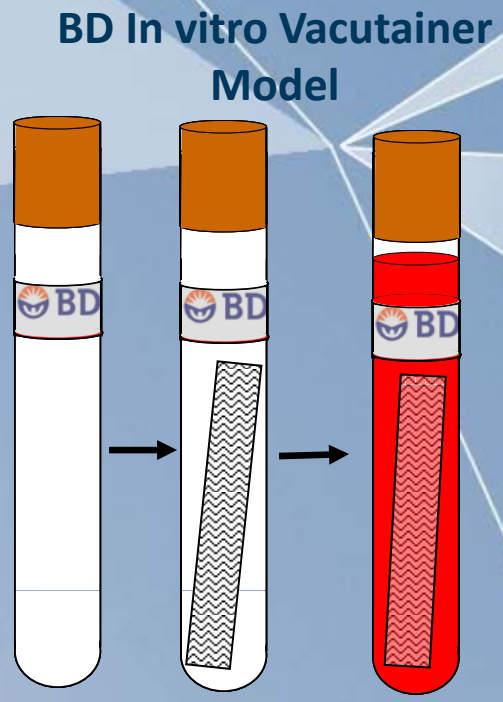


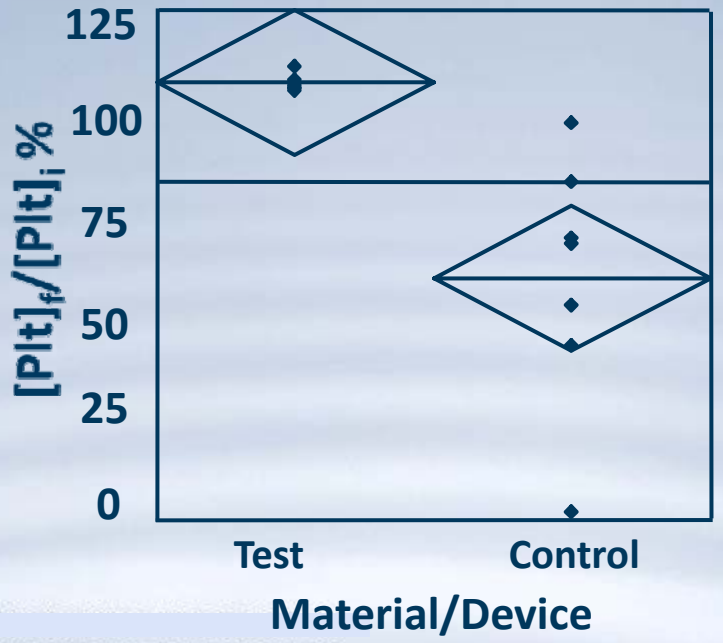




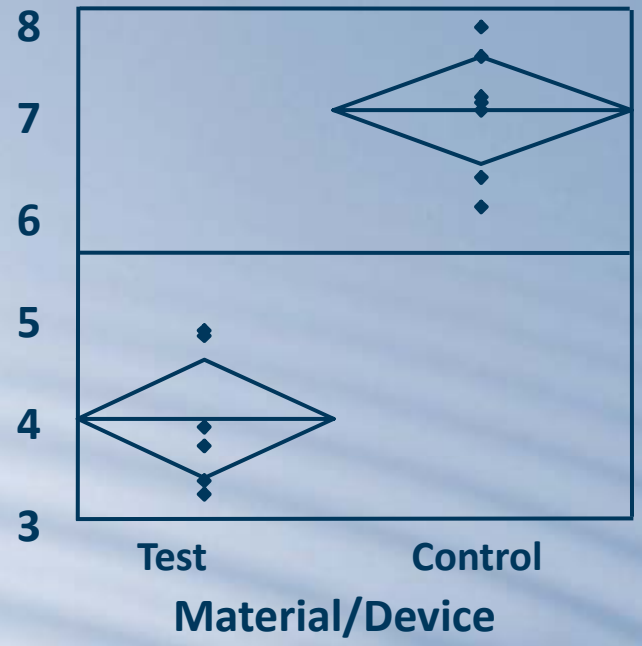
βTG, PF4 released from alpha granules







Beta-TG
Release
ln (ng/mL)



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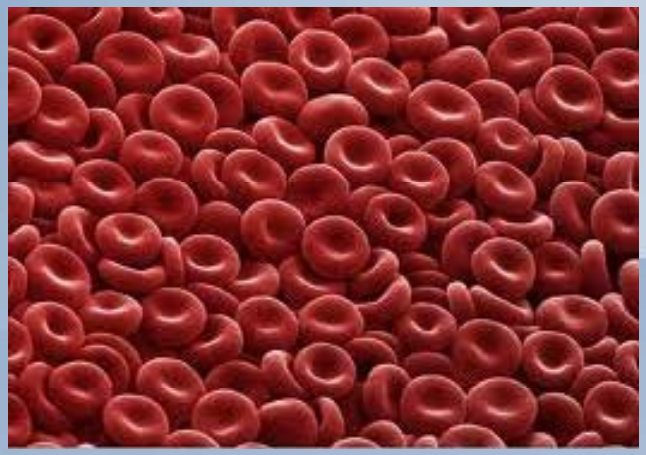

Hemolysis

human

Blood source

rabbit

2013 Round Robin Study
x 10 laboratories

A B C D E F
Material

ASTM direct

ASTM indirect

NIH direct

MHLW indirect

Hemolysis assay

‘Short list’ of tests used to assess interaction with blood

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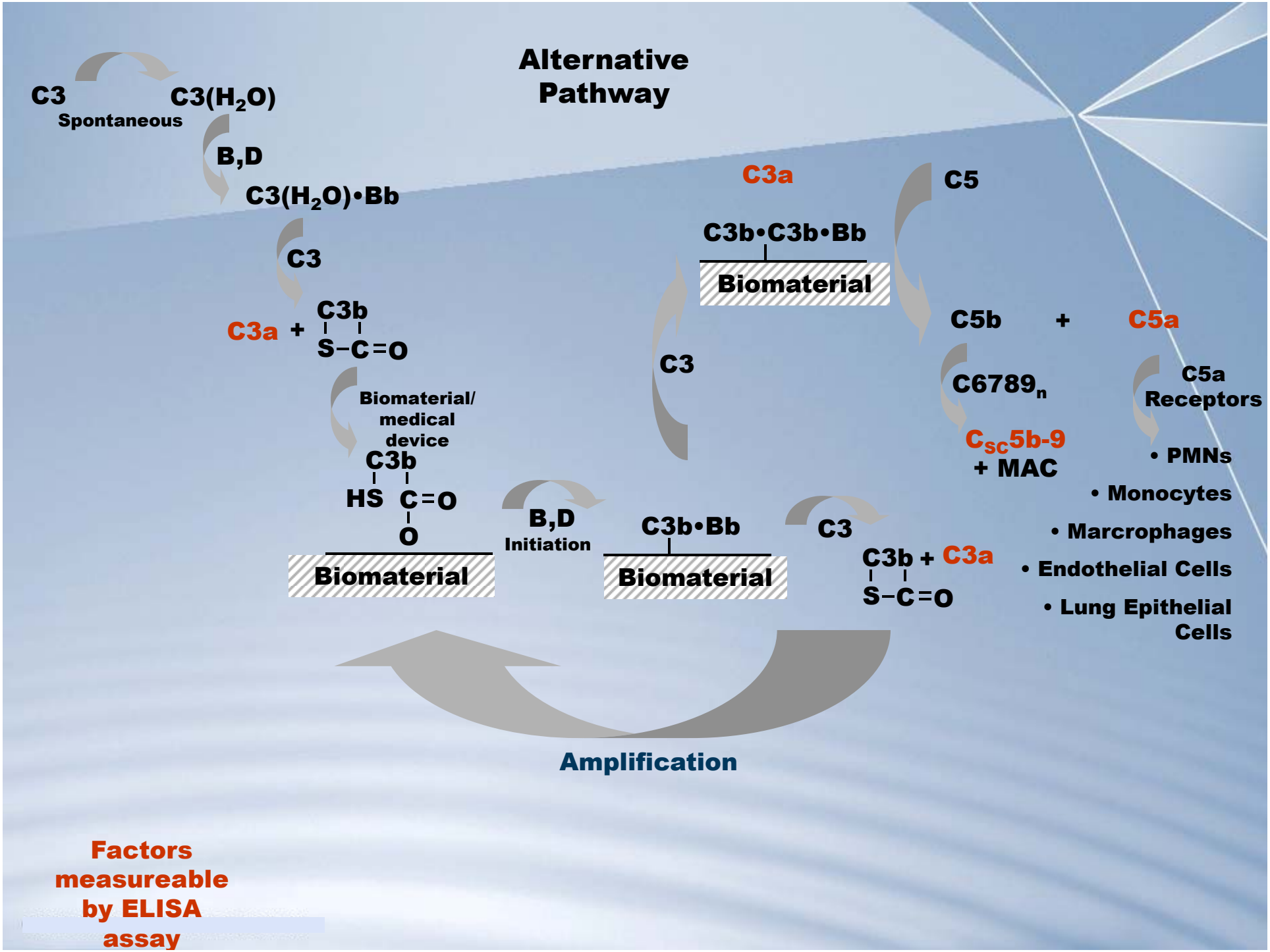
Alternative Pathway
C3
Spontaneous
C3(H2O)
B,D
C3(H2O)•Bb
C3
C3a + C3b
S–C=O
Biomaterial/ medical device
C3b
HS C=O
O
Biomaterial
B,D
Initiation
C3b•Bb
Biomaterial
C3
C3b + C3a
S–C=O
C3a
C3b•C3b•Bb
Biomaterial
C3
C5
C5b + C5a
C6789n
Csc5b-9 + MAC
C5a Receptors
• PMNs
• Monocytes
• Marcrophages
• Endothelial Cells
• Lung Epithelial Cells
Amplification
Factors measureable by ELISA assay

Review of multiple laboratory complement testing methodologies

1. Complement factor(s) measured: CONSISTENT

i. most labs measured C3a and SC5b9
ii. no labs measured C5a (the major clinical factor [1])

2. Blood preparation and anticoagulation use: VARIABLE

i. special commercially-available human serum
ii. fresh citrated human plasma*
iii. fresh human serum
iv. directly-exposed fresh heparinized whole human blood

3. Ratio of test article surface area to blood (whole, plasma or serum) volume: VARIABLE

i. some laboratories specify following ISO10993 Part 12 ratios
ii. some laboratories do not specify a ratio
iii. a variable ratio is used**

* complement activation is calcium-dependent; use of calcium chelators such as sodium citrate or EDTA shut off complement

** complement activation is influenced by surface area (SA); standardized and reported SAs are important to inter- and intra-laboratory interpretation of results.

[1] Johnson R J (2013), 'The complement system', Biomaterials Science: An Introduction to Materials in Medicine , 3rd Ed, Oxford, UK, Elsevier Academic Press, 533–545

Review of multiple laboratory complement testing methodologies (continued)

4. Use of controls: ~ CONSISTENT

i. common use of a negative biomaterial control such as polypropylene and a positive biomaterial control such as latex or cellulose
ii. some use of a liquid negative control such as saline and a positive liquid control such as cobra venom
iv. consistent use of negative and positive controls provided in the commercially-available kits

5. Standard curve preparation/dilutions: CONSISTENT

i. laboratories used different dilutions to generate a standard curve that captured most levels of test and control samples, e.g., 1:100, 1:200, 1:1000, and 1:10,000

6. Incubation period for test articles and controls: VARIABLE

i. single point: labs reporting use of 60- or 90- minute incubation periods
ii. multiple point: one lab used 30- and 60- and 90-minute incubation periods

7. Incubation time following addition of chromogenic substrate: ~ CONSISTENT

i. 15-30 minute incubations periods were reported

8. Test sample evaluation: INCONSISTENT

i. most laboratories made a statistical comparison of test sample results to positive and negative biomaterial and/or liquid controls
ii. some laboratories included a comparison to historical values, results on a predicate device, and/or a special mathematical formula involving negative and positive controls, as part of the final assessment


Thank you