

Biological Activity of Adsorbed Proteins
And Biocompatibility

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Outline

- 1. Biological activity of adsorbed proteins: role in biomaterials.**
- 2. Biochemical basis of the biological activity of adsorbed proteins: the adhesion proteins and their receptors, the integrins.**
- 3. Major mechanisms affecting the biological activity of adsorbed proteins: affinity and molecular potency; some examples.**
- 4. Affinity variations in adsorption of adhesion proteins: The challenge of fibrinogen and nonfouling materials**

1. Biological activity of adsorbed proteins: examples for biomaterials

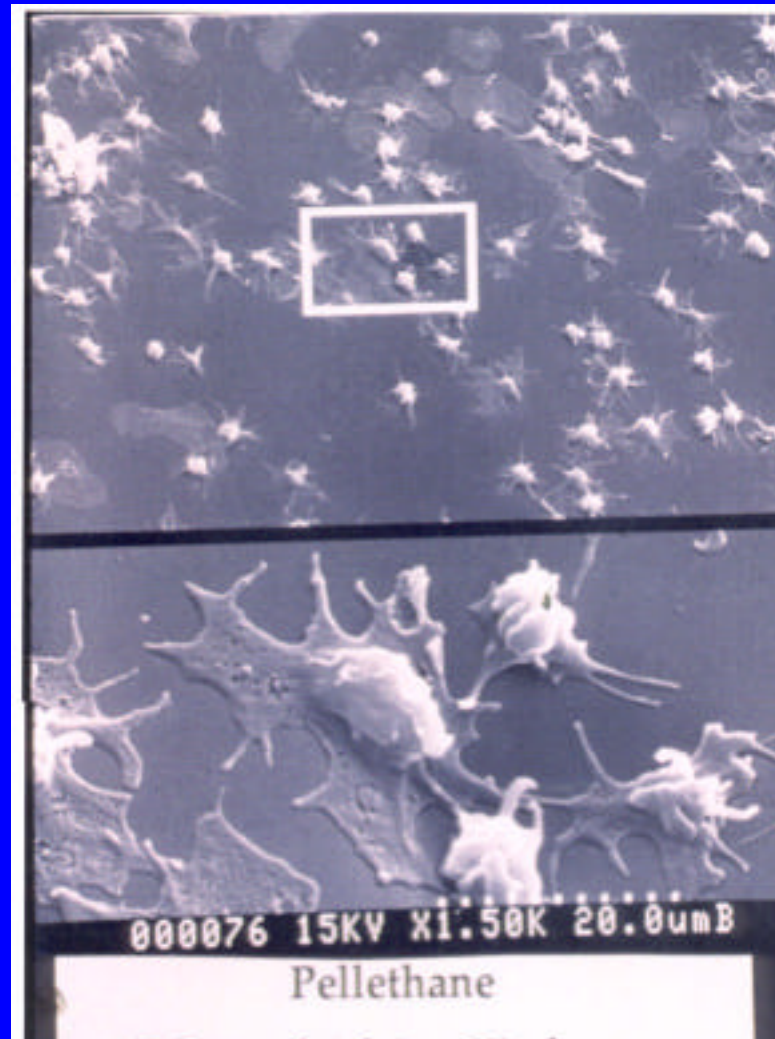
- **Platelet adhesion and blood clotting on foreign surfaces**
- **Monocyte/macrophage adhesion and the foreign body reaction**

Why we need more blood compatible biomaterials



The photo above shows results of an in vivo study in which uncoated and heparin-coated polyurethane rods were implanted for one hour in the right and left external jugular veins of non-anticoagulated dogs.

**Biological activity of adsorbed proteins:
The example of platelet adhesion and activation**



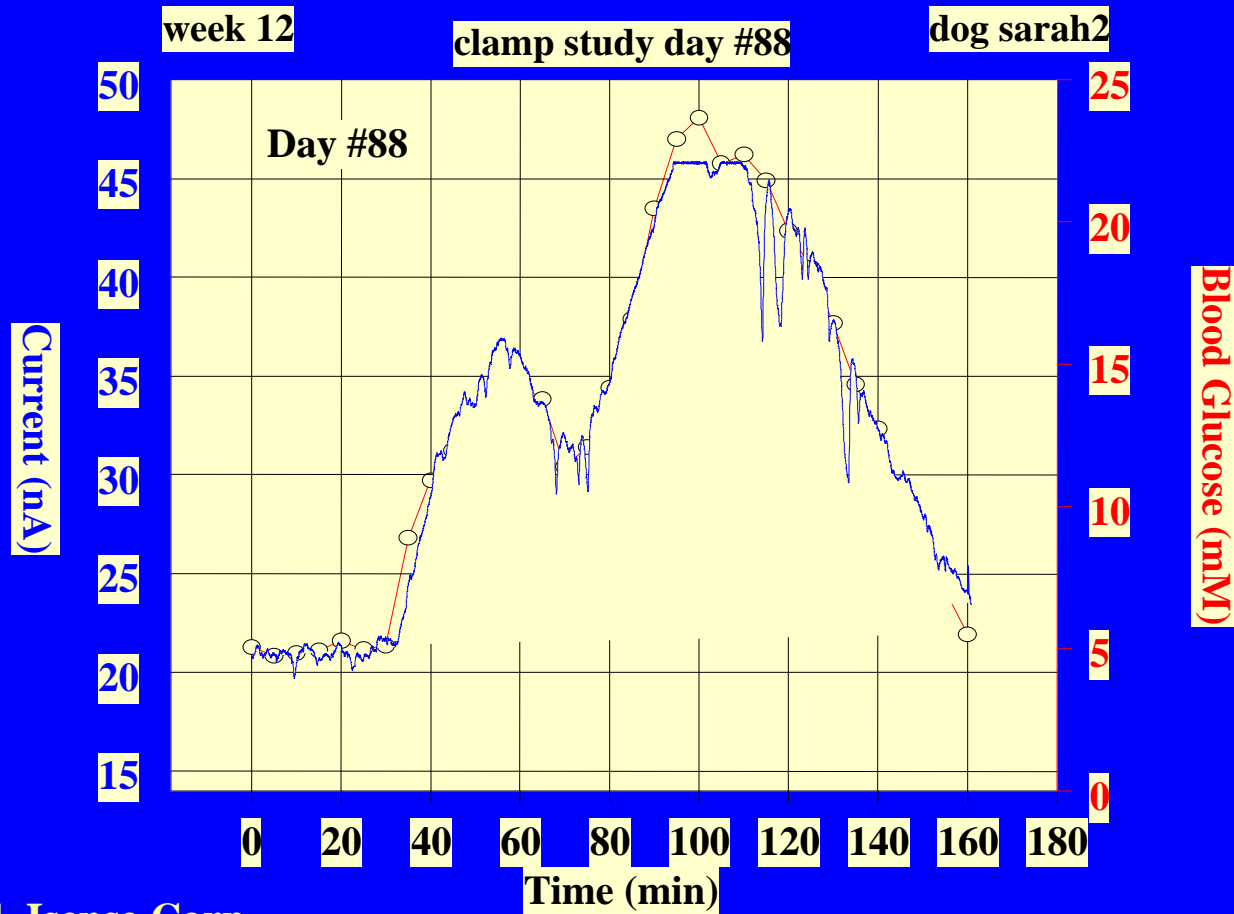
Design Criteria for Blood Compatibility: Platelet adhesion is a procoagulant event

Effect of Adherent Platelets on Clotting Times of Recalcified Plasma³.

	Platelets	No Platelets	Platelets with Annexin V	No Platelets with Annexin V
Glass	5.6+/-0.4	8.7+/-0.5	7.0+/-0.6	13.6+/-0.3
TCPS	9.3+/-0.3	21.4+/-1.1	>60	>60
Thermanox [®]	11.2+/-2.8	>60	n.a.	n.a.

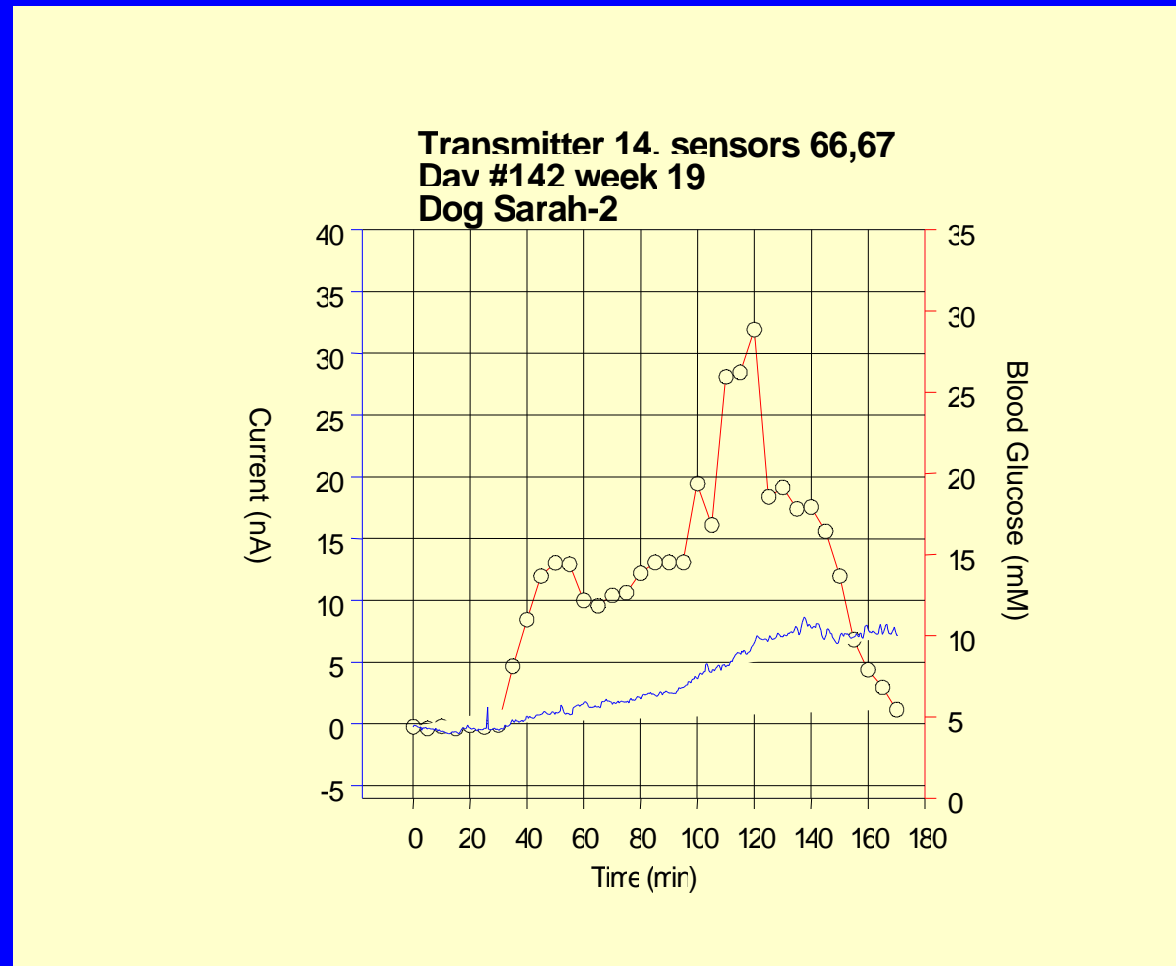
³Times reported are in Minutes. Mean +/- stdev (n=4). TCPS is Falcon tissue culture polystyrene. Thermanox[®] is polyethyleneterephthalate (PET). n.a. means, data not available.

Why we need to reduce the soft tissue reaction: The example of glucose sensors



From K. Ward, Isense Corp

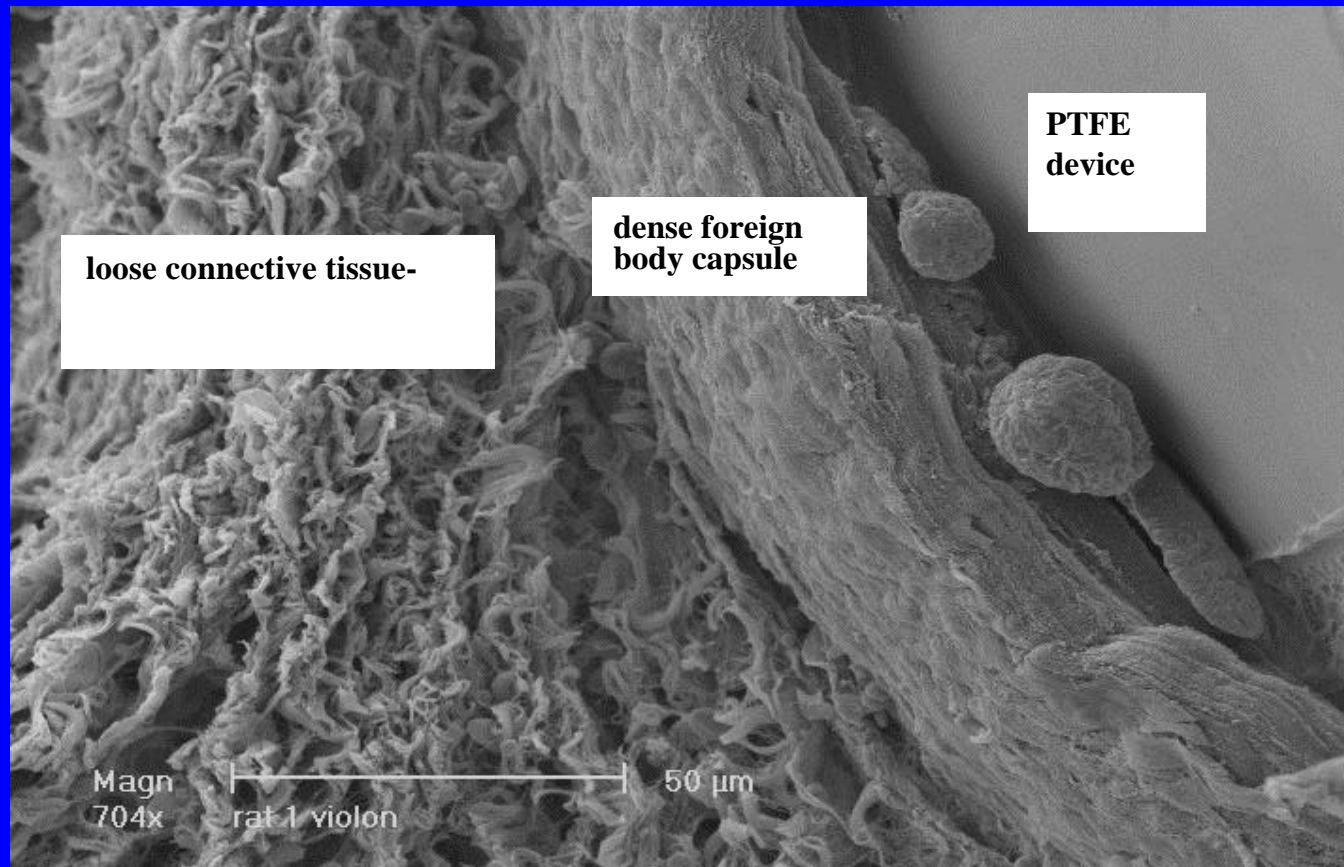
Why we need to reduce the soft tissue reaction: The example of glucose sensors



From K. Ward, Isense Corp

Why we need to reduce the soft tissue reaction: The example of glucose sensors

SEM of tissue surrounding non-recording subcutaneous device in rat: 6 weeks after implant



From K. Ward, Isense Corp

2. Biochemical basis of the biological activity of adsorbed proteins:
The adhesion proteins and their receptors, the integrins.

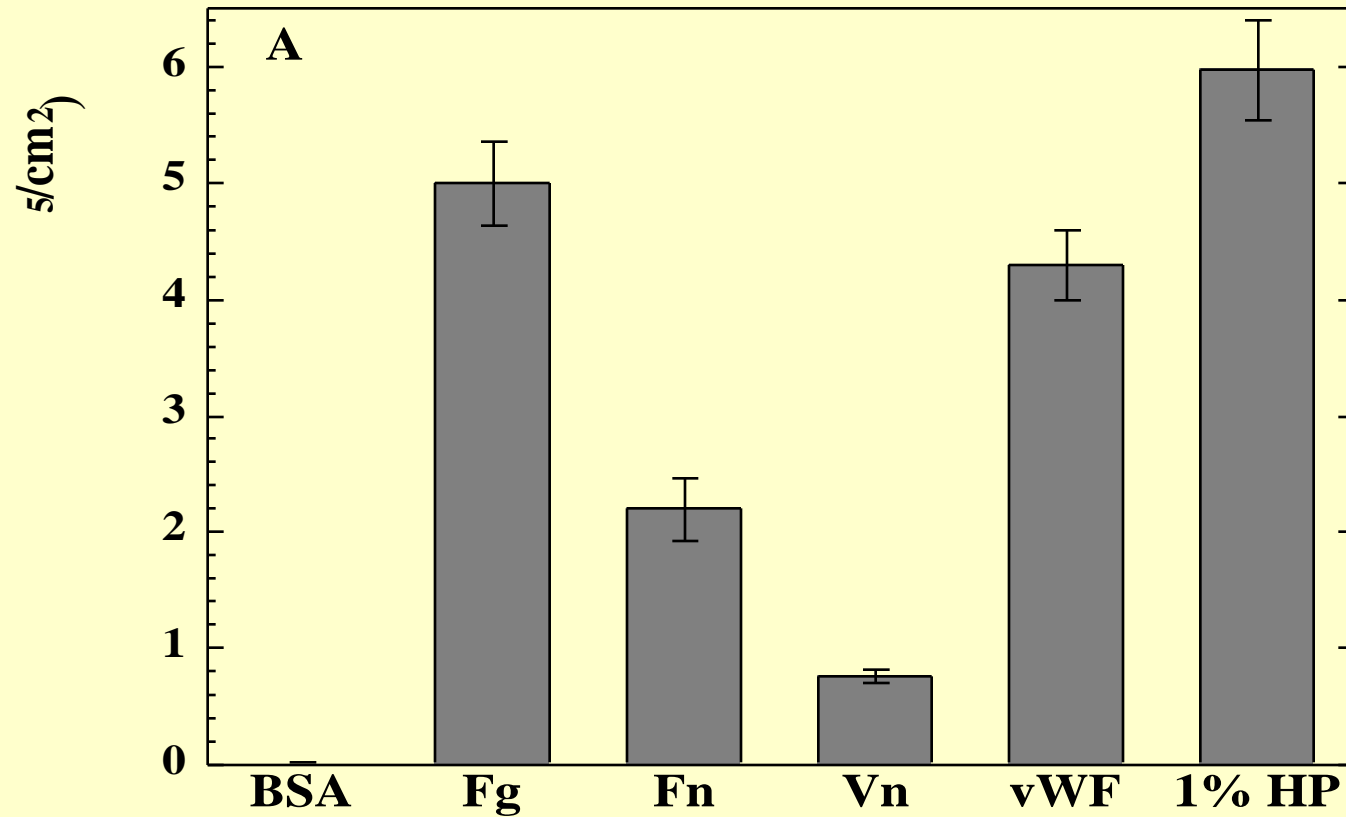
- **Adhesion proteins in plasma: Fg, Fn, Vn, and vWf;**
- **Preadsorption or selective depletion shows their role**
- **Adhesion receptors: the integrins in adhesion and signaling**

Biochemical basis of the biological activity of adsorbed proteins:
Preadsorption effects on cell adhesion define
“Adhesion” and “Blocking” proteins

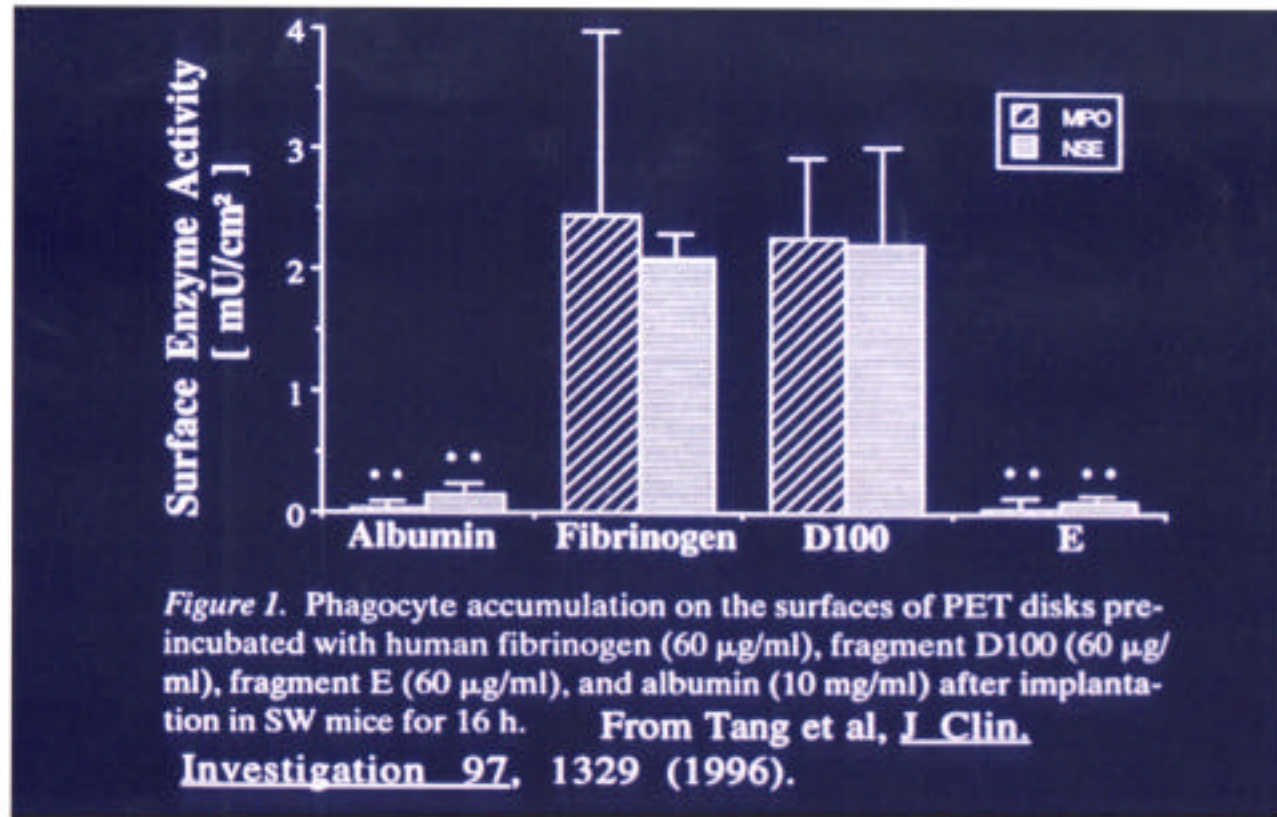
- Preadsorption of certain purified proteins such as Fg, Fn, Vn, & vWf greatly increases cell attachment, defining them as adhesion proteins.
- Preadsorption of other proteins such as BSA prevents or greatly reduces cell attachment, defining them as “blocking” proteins.

Biochemical basis of the biological activity of adsorbed proteins:
Effect of Preadsorbed Proteins on Platelet Adhesion to Polystyrene

Platelet Adhesion (X 10⁵/cm²)



Biochemical basis of the biological activity of adsorbed proteins:
Fibrinogen supports phagocyte accumulation on intraperitoneal implants



Biochemical basis of the biological activity of adsorbed proteins:
Selective depletion effects also show activity of adhesion proteins

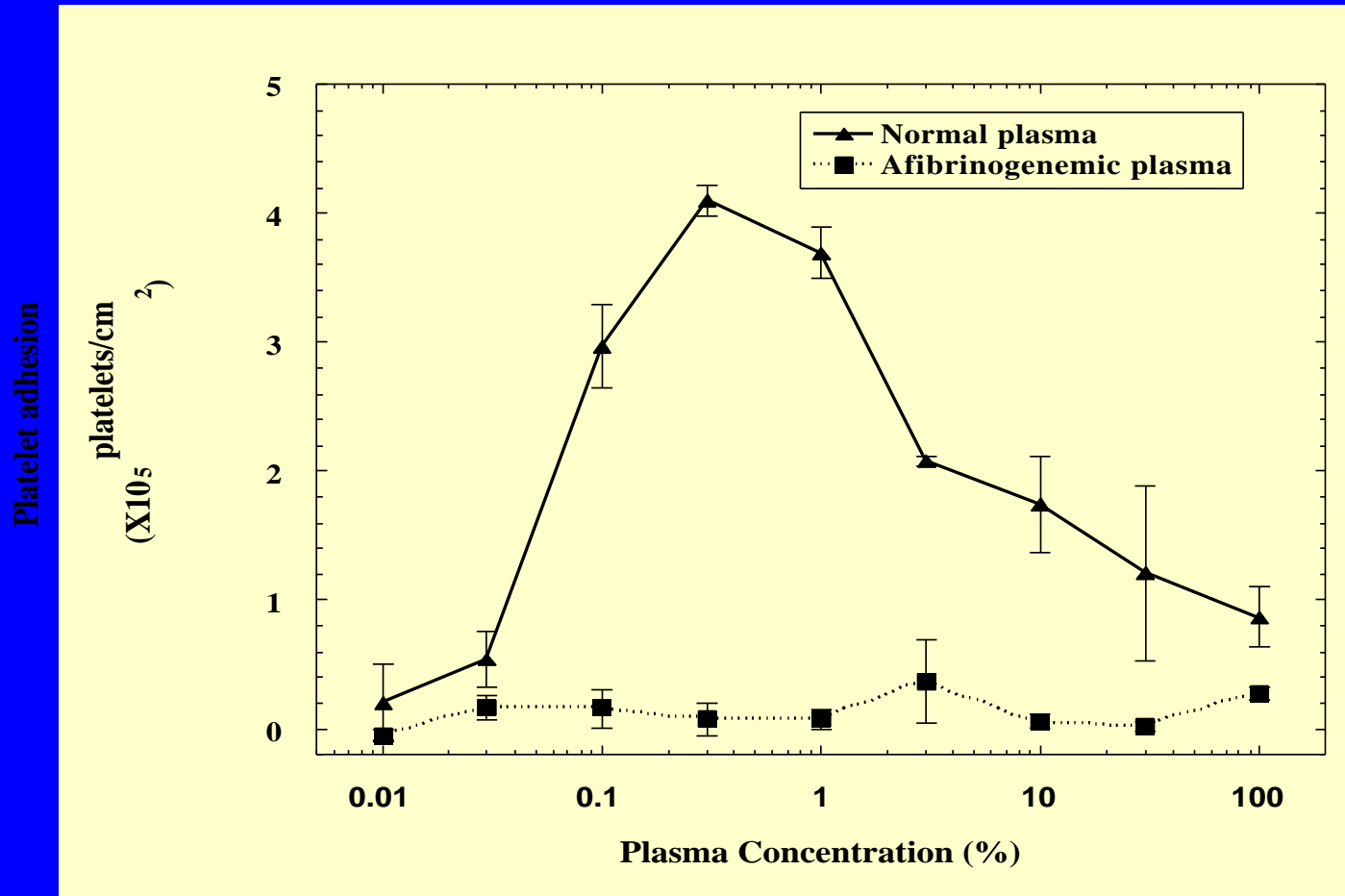
Removal of one protein from plasma or serum used to preadsorb a surface can cause cell attachment to greatly decrease, while addition of the depleted protein restores adhesion.

Examples:

- 1. Afibrinogenemic plasma or serum for selective removal of Fg: Fg removal from plasma affects platelet adhesion to a fairly wide variety of surfaces.**
- 2. Gelatin or immunoadsorbent columns for selective removal of Fn or Vn: Vn rather than Fn is the key protein in mediating adhesion of several kinds of cells to some surfaces.**
- 3. Ancrod depletion of Fg in mice: Fg is key in acute phase FBR.**

Biochemical basis of the biological activity of adsorbed proteins:
Selective depletion effects also show activity of adhesion proteins

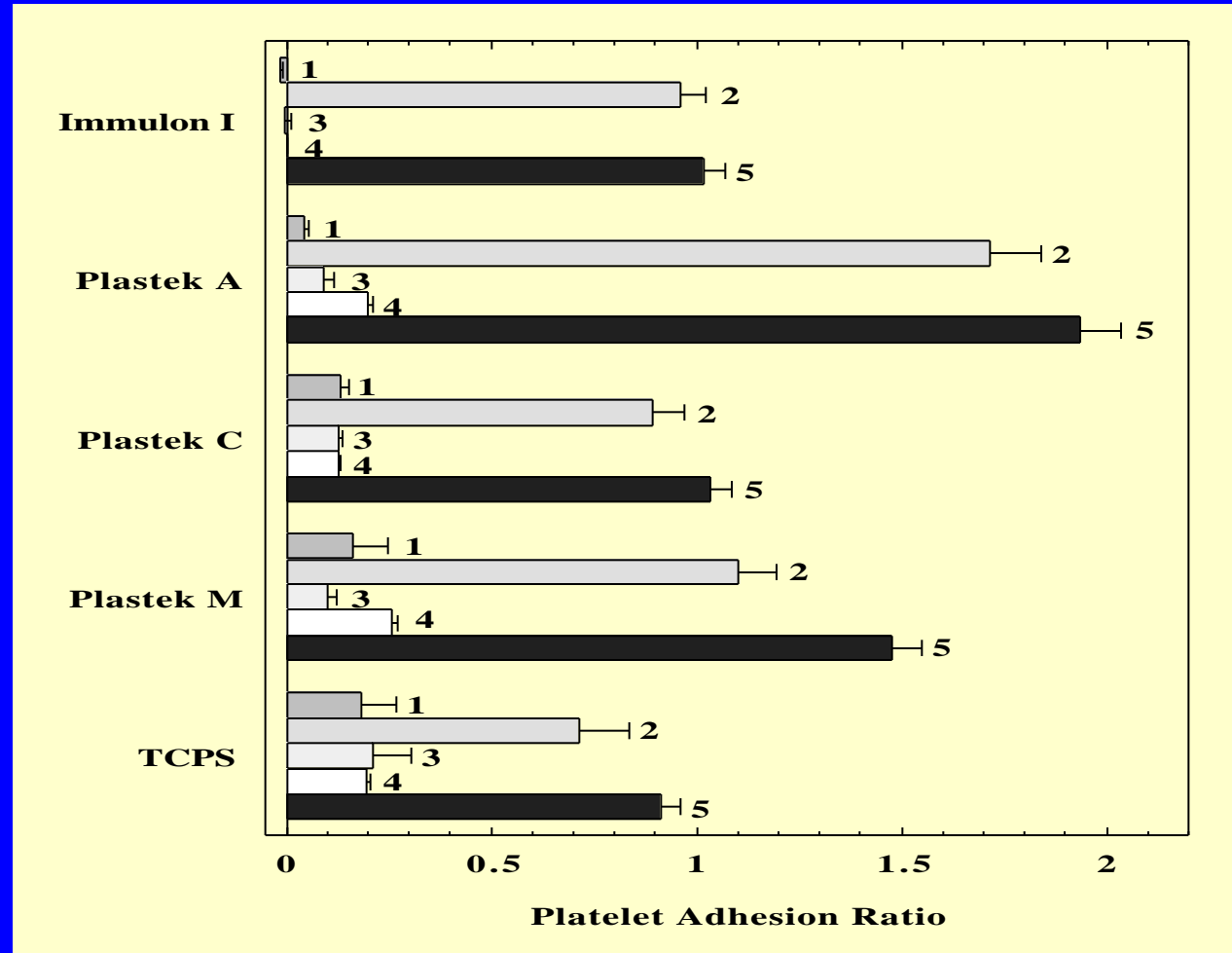
Platelet adhesion to Immulon I[®] preadsorbed with normal or afibrinogenemic plasma.



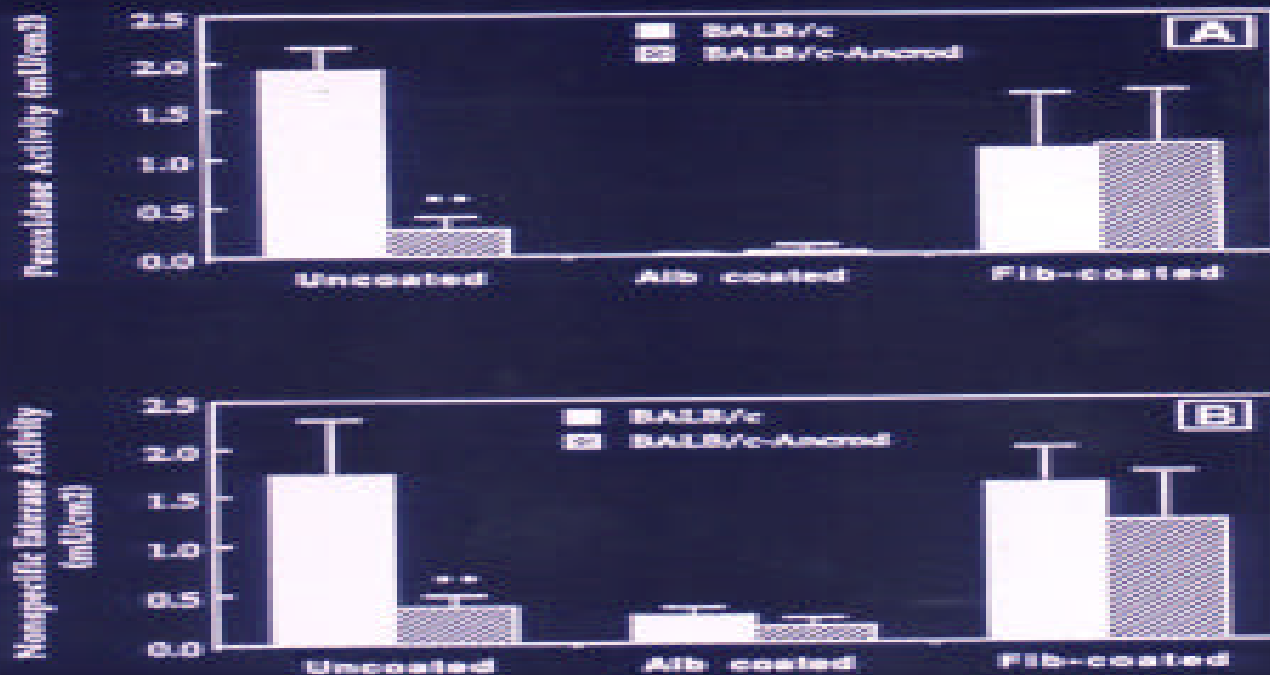
Biochemical basis of the biological activity of adsorbed proteins:
Selective depletion effects also show activity of adhesion proteins

Platelet adhesion to surface preadsorbed with serum replenished with Fg, Fn, or vWF.

1. 1% serum
2. 1% serum + Fg
3. 1% serum + Fn
4. 1% serum + vWF
5. 1% serum + all.



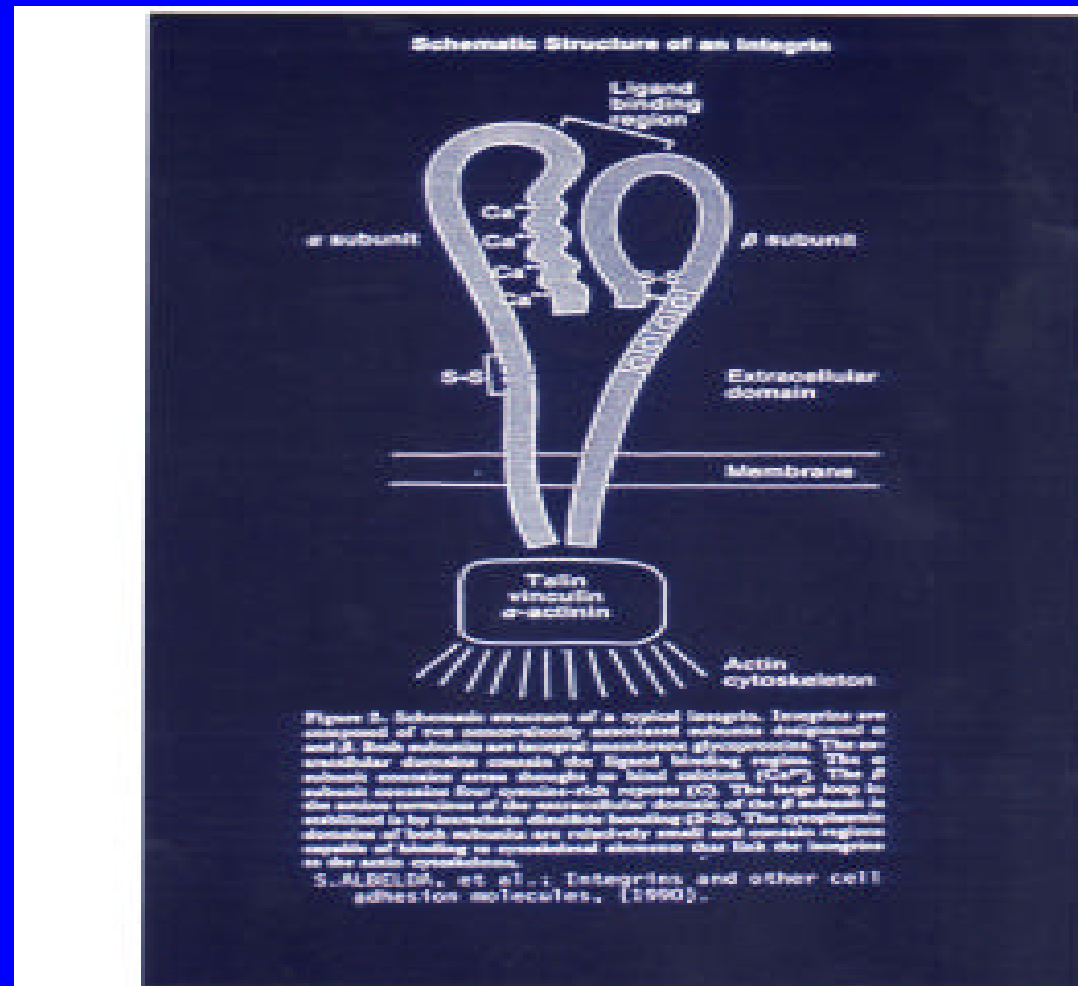
Biochemical basis of the biological activity of adsorbed proteins:
Selective depletion effects show activity of adhesion proteins
Fg removal greatly decreases phagocyte accumulation on ip implants



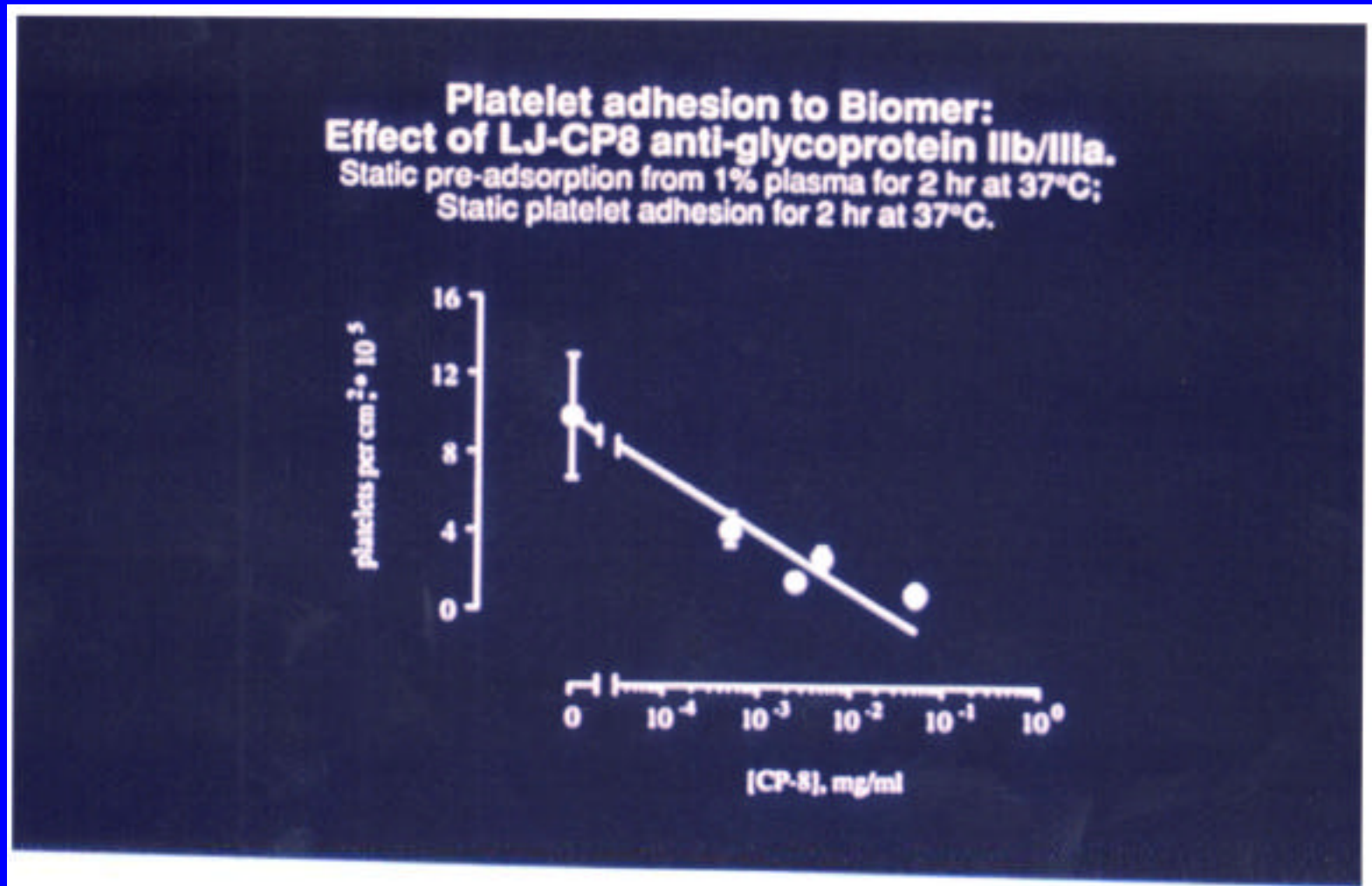
Role of fibrinogen in phagocyte accumulation on the surfaces of PET disks implanted intraperitoneally in mice shown by anored depletion of fibrinogen.

Tang and Eaton, *J. Exp. Med.*, 178, 2147 (1993)

Biochemical basis of the biological activity of adsorbed proteins: **Integrin Cell Adhesion Receptors**



Biochemical basis of the biological activity of adsorbed proteins:
Antibody to Adhesion Receptor GPIIb/IIIa
Inhibits Platelet Adhesion to a Biomaterial

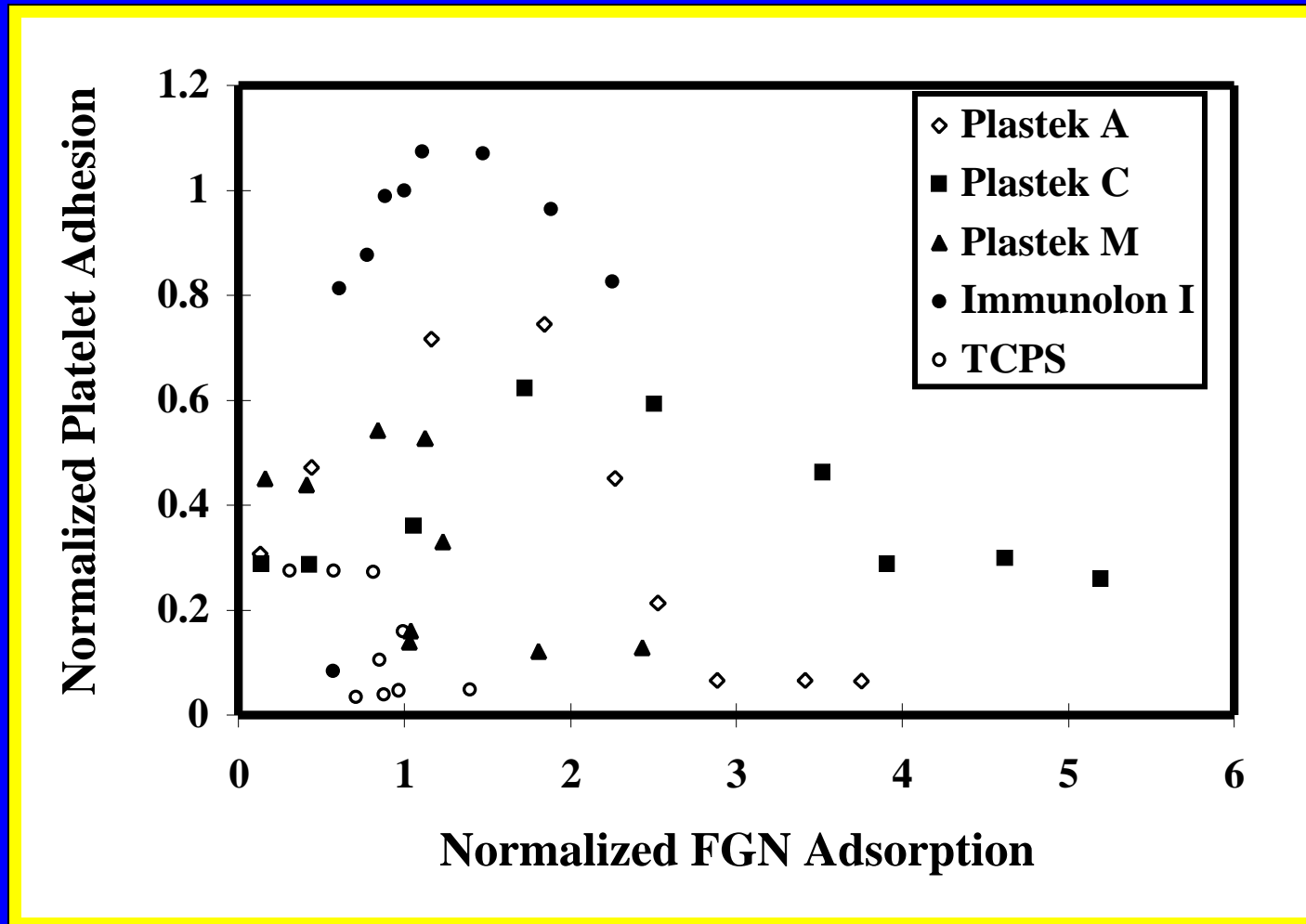


3. Major mechanisms affecting biological activity of adsorbed proteins: **Affinity and Molecular Potency**

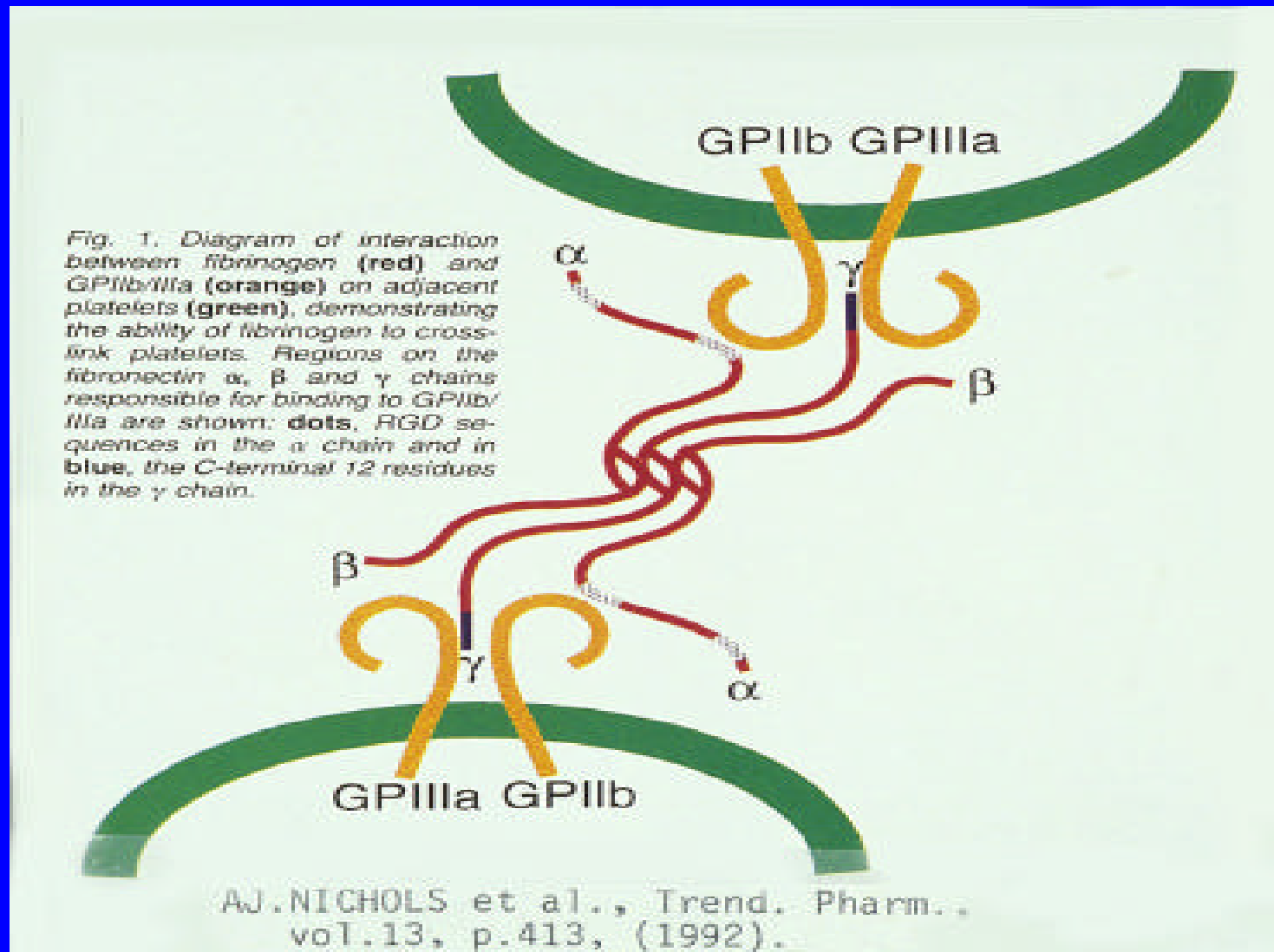
The major mechanisms affecting biological activity of adsorbed adhesion proteins are:

- Affinity of the adhesion protein for a surface, affecting how much of the proteins is present on a particular surface;**
- Molecular potency, defined as the degree to which the adsorbed adhesion protein expresses its biological activity.**

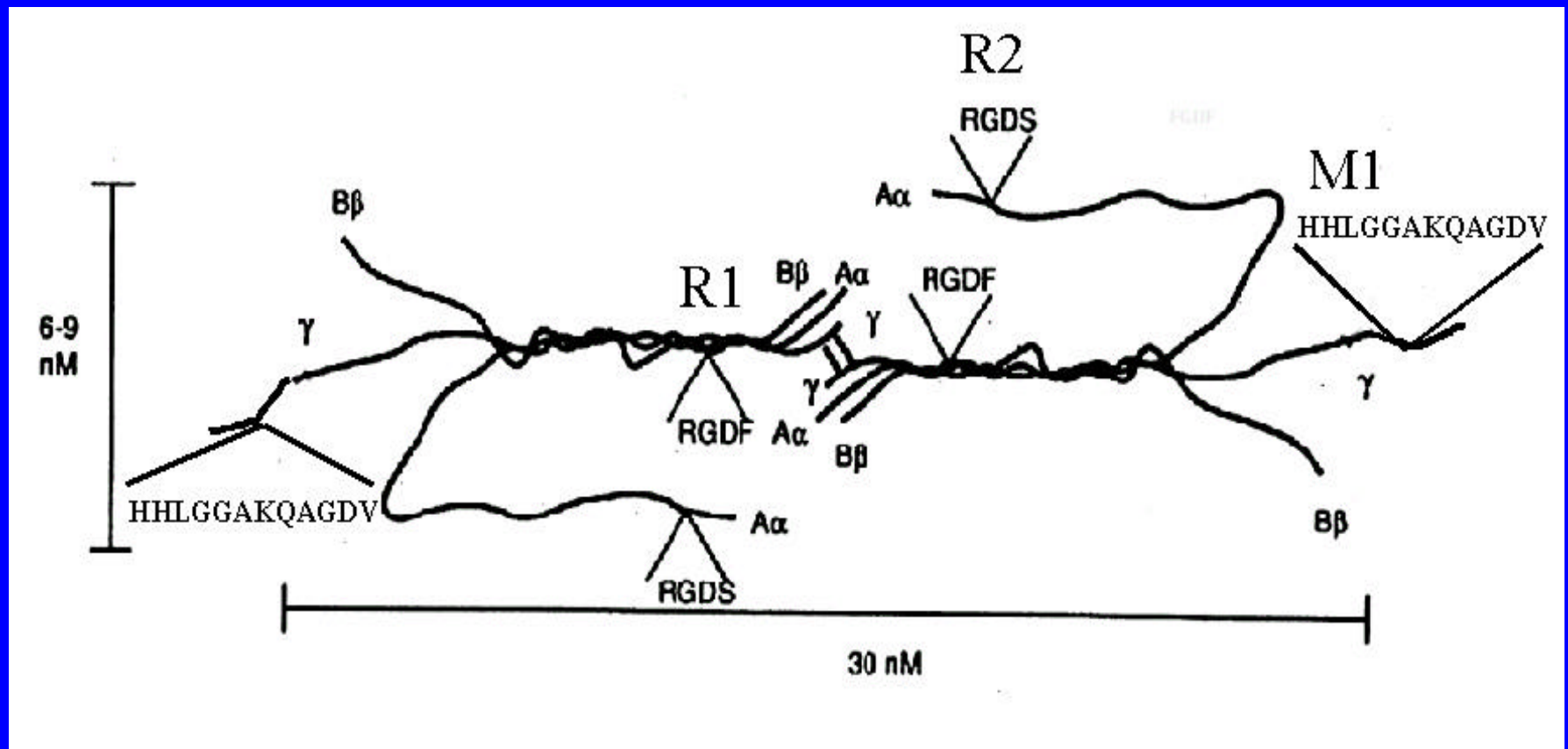
**An example of the role of molecular potency:
Platelet adhesion vs.. Fg adsorption: Why no correlation?**



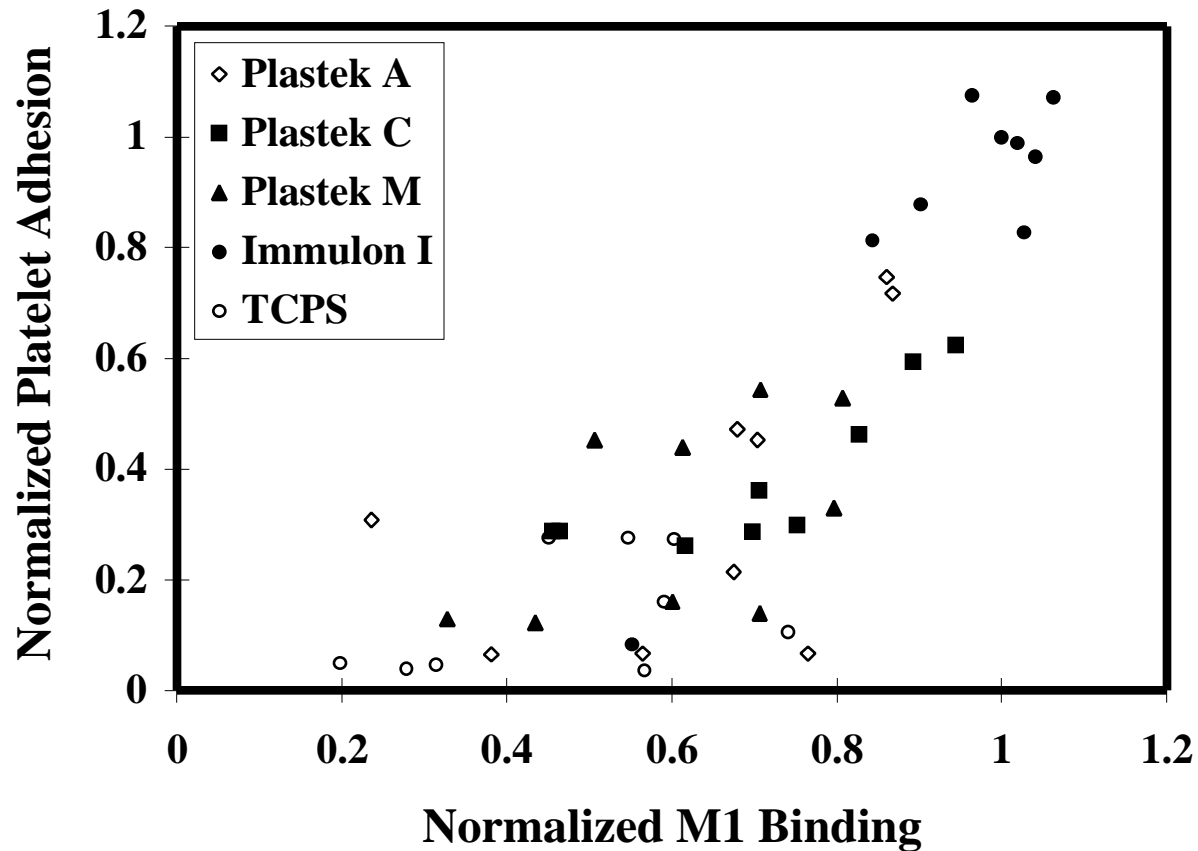
Fibrinogen and GP IIb/IIIa in platelet aggregation



Platelet Binding Sites in Fibrinogen



Molecular Potency Shown by Antibodies: Platelet adhesion vs. M1 antibody binding



Affinity of the adhesion proteins

Relatively little direct data exists on this fundamental issue.

However, competitive adsorption studies from binary mixtures, and adsorption studies from serum or plasma, suggest that the relative affinity of adhesion proteins is as follows for most surfaces:

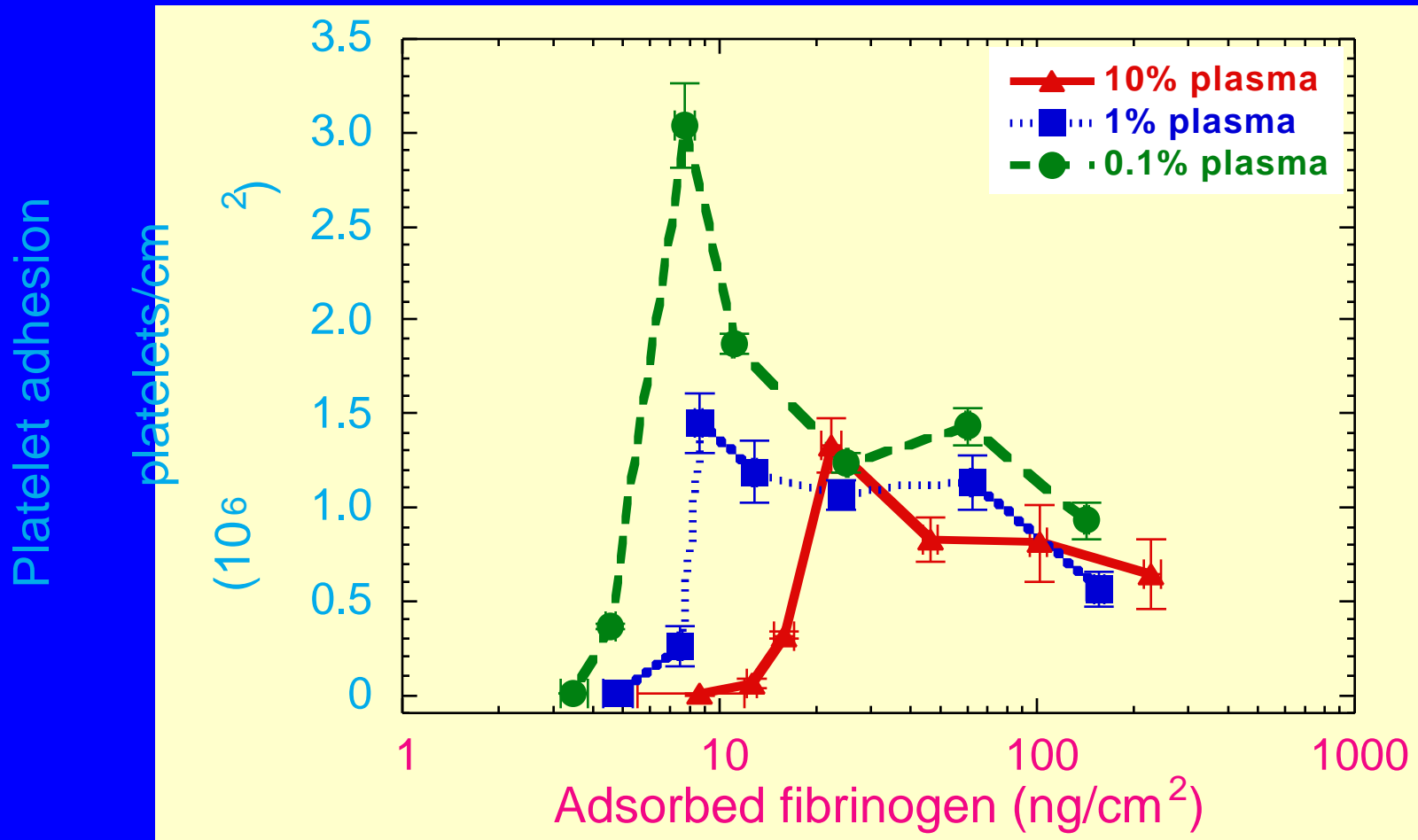
Fibrinogen>Vitronectin>Fibronectin>>Albumin

"Relative affinity" denotes how well a given protein competes for a surface against another protein. In binary mixtures of two proteins, equal weight concentrations of an adhesion protein and a competing protein would be expected to result in equal surface concentrations of the two proteins unless one has a higher affinity relative to the other, and so binary mixture studies in which these are varied is one way to estimate competitive or relative affinity.

Affinity variations in adsorption of adhesion proteins: Nonfouling materials: The Challenge of Fibrinogen

- 1. What is “The Challenge of Fibrinogen”?**
- 2. How successfully is this challenge being met?**

Design Criteria for Blood Compatibility: Effect of Fg Repletion on Platelet Adhesion to Polystyrene Preadsorbed with Afibrinogenemic Plasma

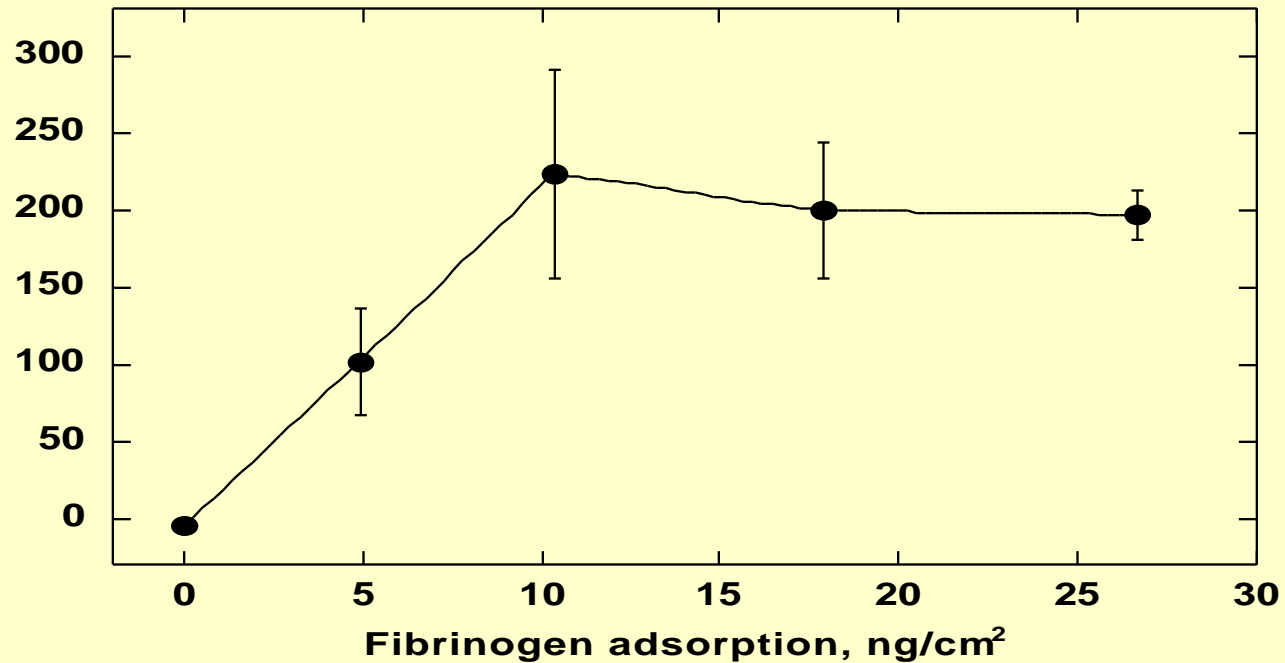


Design Criteria for Blood Compatibility:

Platelet adhesion from platelet/red cell suspensions flowing at 500 sec^{-1} to tubular polyethylene preadsorbed with Fg from 10% serum.

Platelet adhesion, #/cm

$\times 10^3$

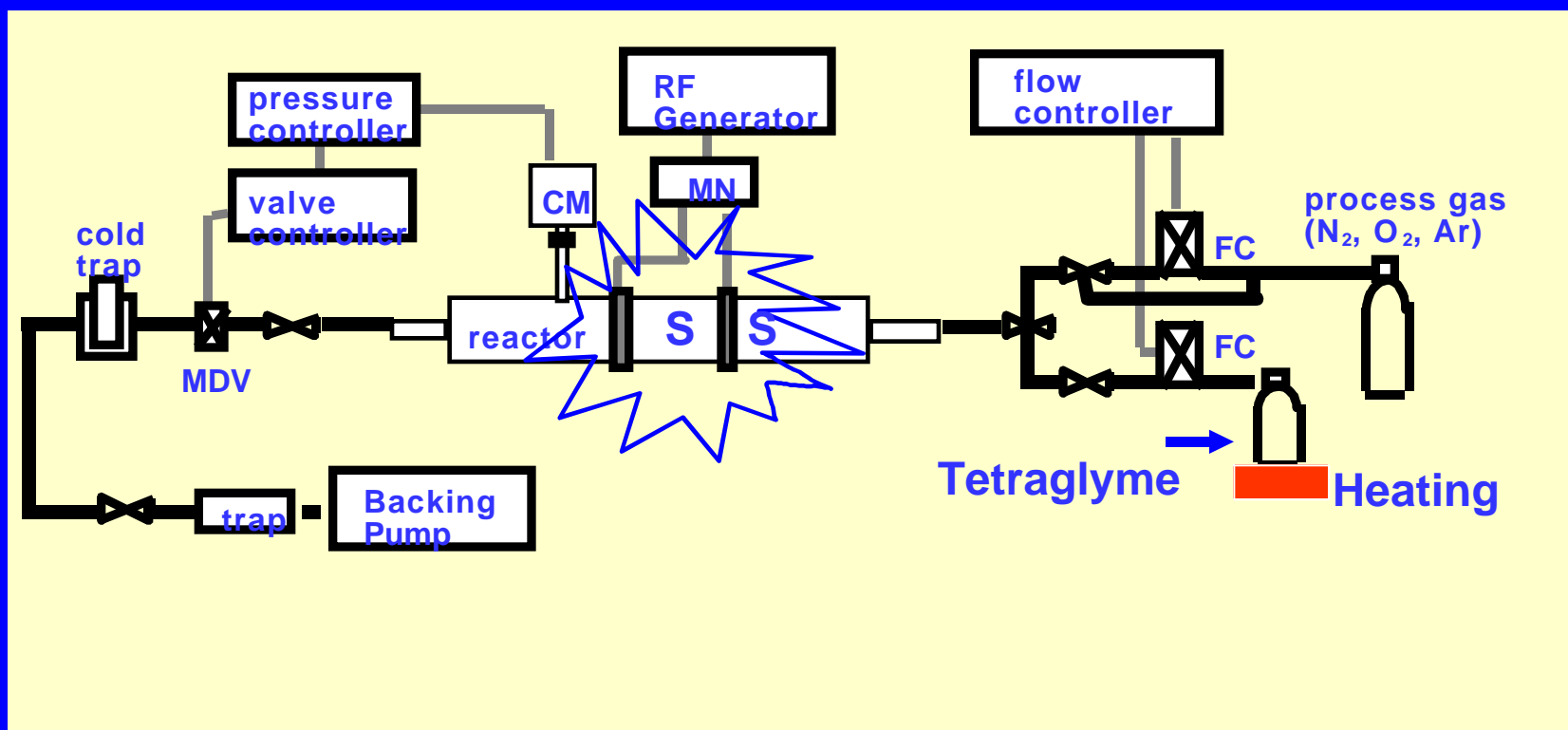


Affinity variations in adsorption of adhesion proteins: Nonfouling materials: The Challenge of Fibrinogen

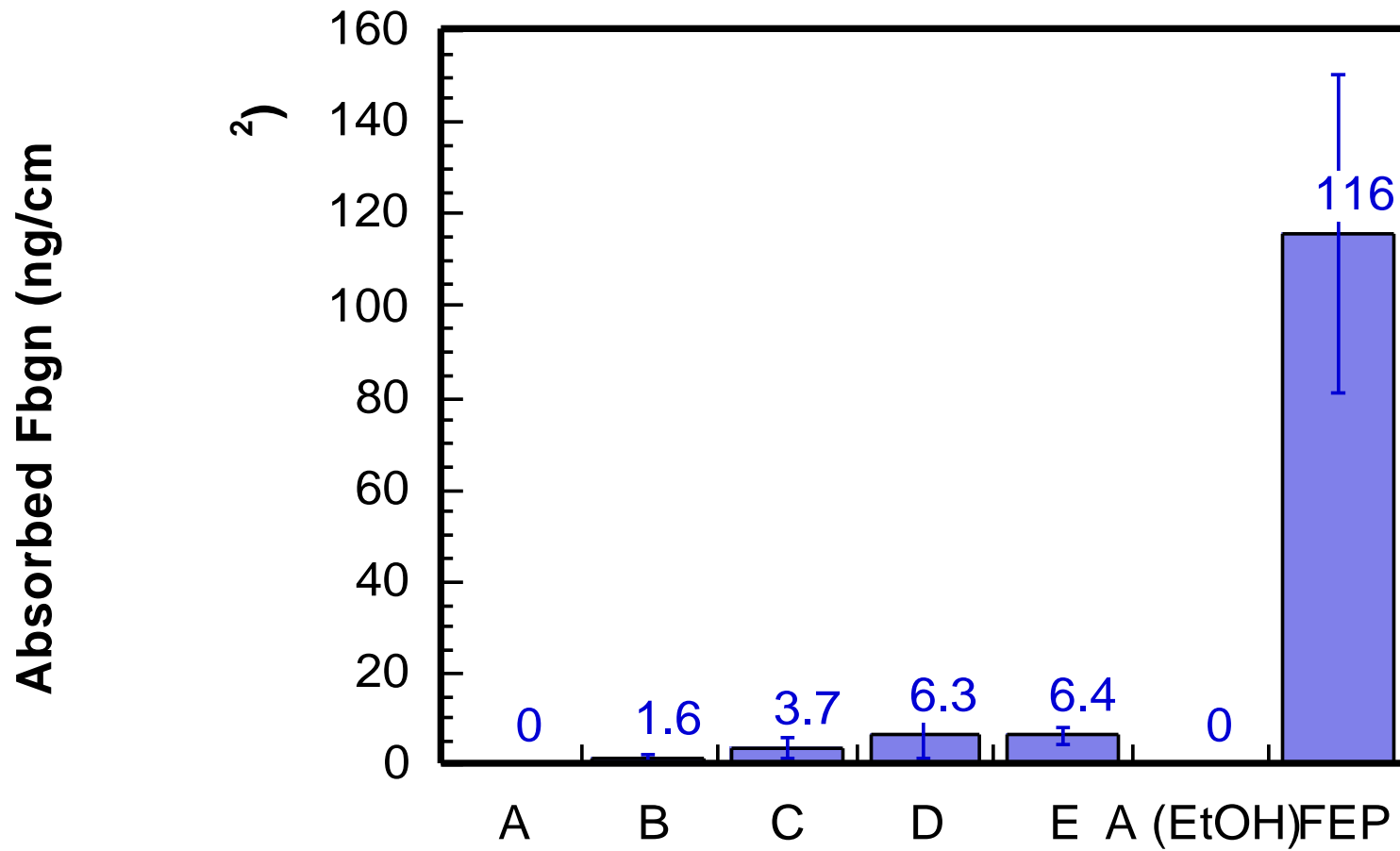
How successfully is this challenge being met?

- 1. Horbett, Ratner et al: Fg reduced to less than 1 ng/cm² for tetraglymes.**
- 2. Park et al:**
 - a. less than 20 ng/cm² for grafted Pluronics on glass (control 470 ng/cm²);**
 - b. 10-250 ng/cm² for grafted PEO-PB-PEO triblock on glass, silanized glass, and ePTFE.**
- 3. Malmsten and Muller: “nearly zero”.**
- 4. Liu et al: down to 400 ng/cm² for PEO grafted to PEUs(5800 on control).**

RF Glow Discharge Plasma Deposition of Tetraglyme: $\text{CH}_3\text{O}-(\text{CH}_2-\text{CH}_2-\text{O})_4-\text{CH}_3$

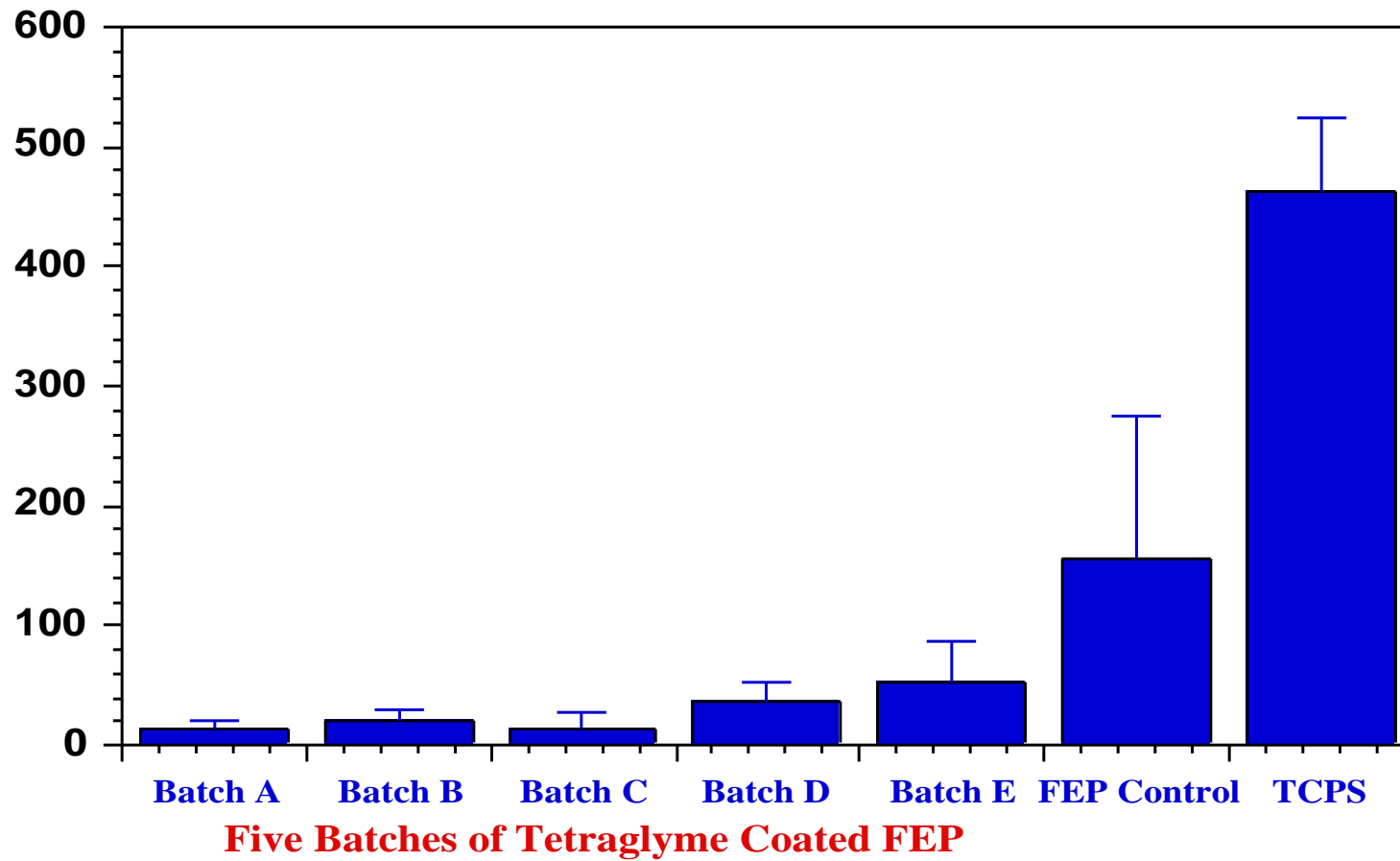


Fibrinogen Adsorption to Plasma Polymerized Tetraglyme on FEP



Monocyte Adhesion to Tetraglyme Coated FEP (2 hr)

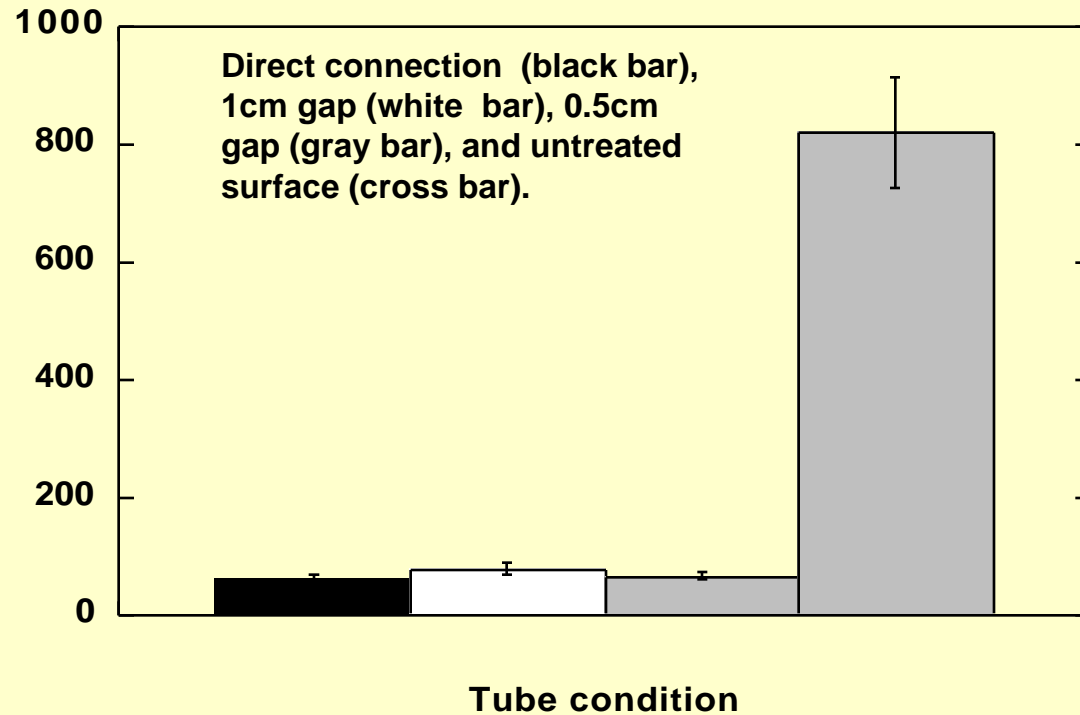
2



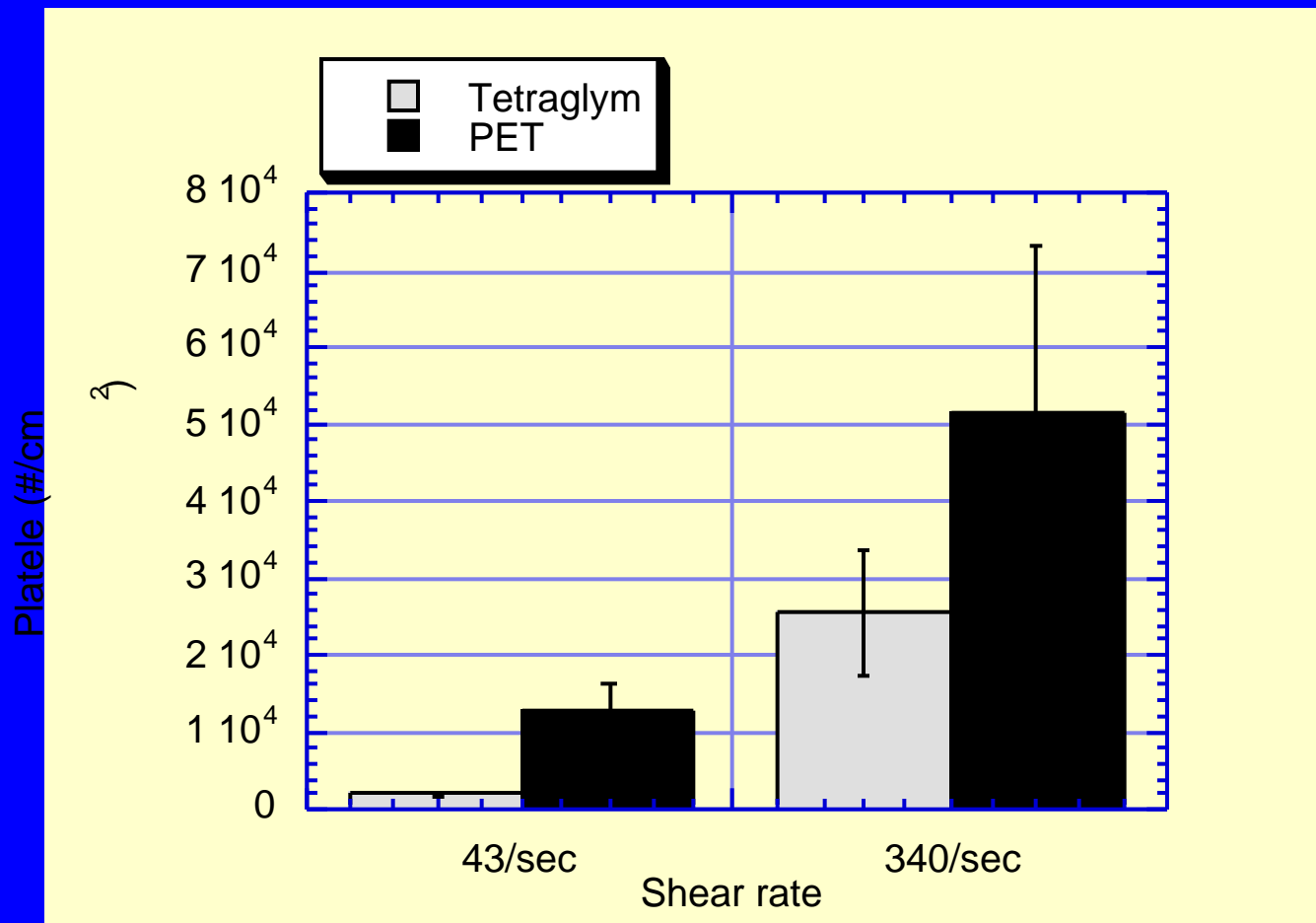
Ultralow fibrinogen adsorption biomaterials: Fg Adsorption to Plasma Polymerized Tetraglyme Coated PE Tubes.

Fibrinogen adsorption, ng/cm

2



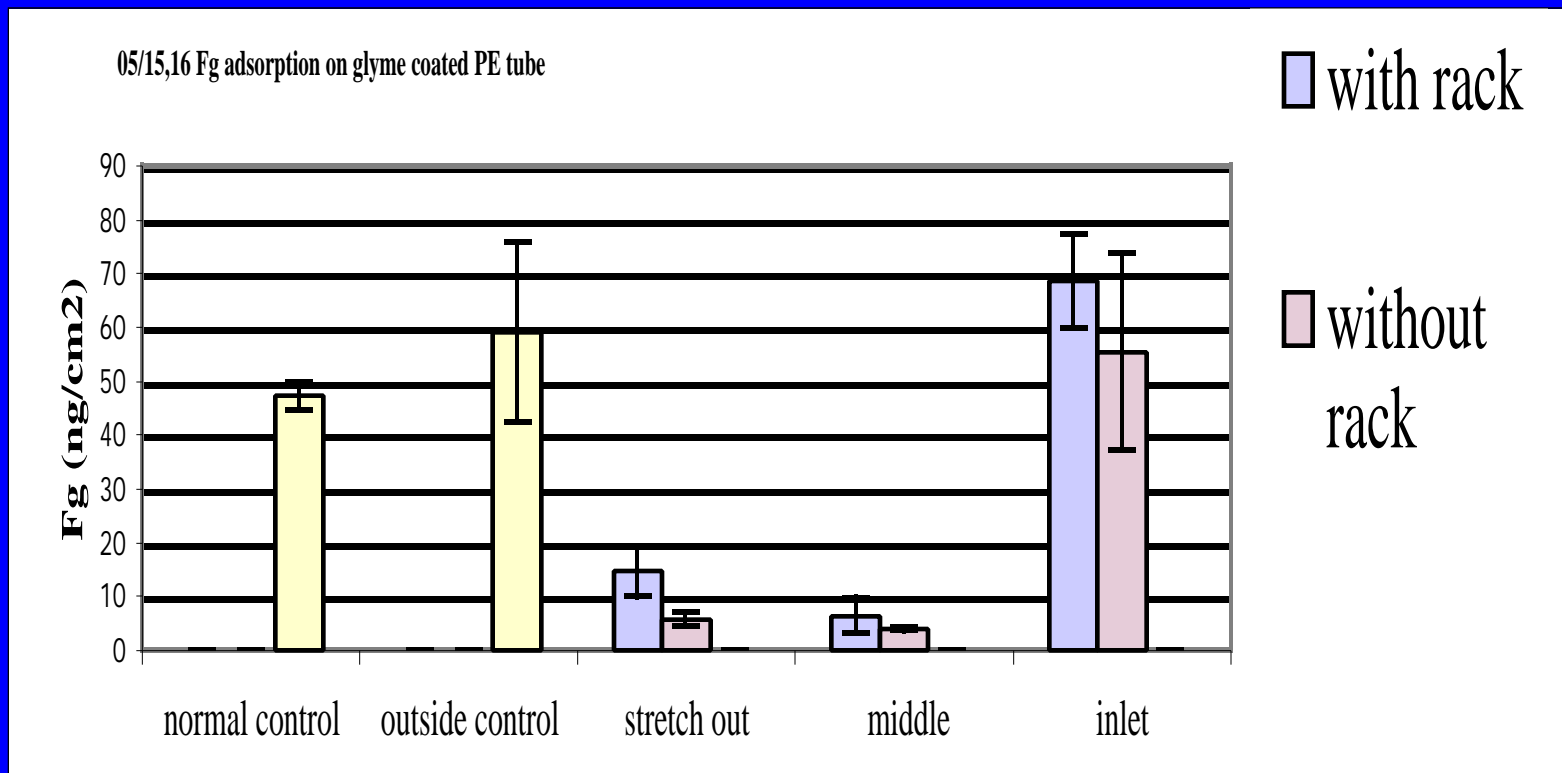
Ultralow fibrinogen adsorption biomaterials: Platelet adhesion to tetraglyme coated PET samples with 0.03 mg/ml Fg.



Ultralow fibrinogen adsorption biomaterials: Fg adsorption to plasma deposited tetraglyme on PE tubes

Recent studies with modified conditions: Effect of location in tube and tube holder

Reactor conditions: Initial pressure of 100mT of tetraglyme plasma; 80W 1min, followed by 10 W for 10min

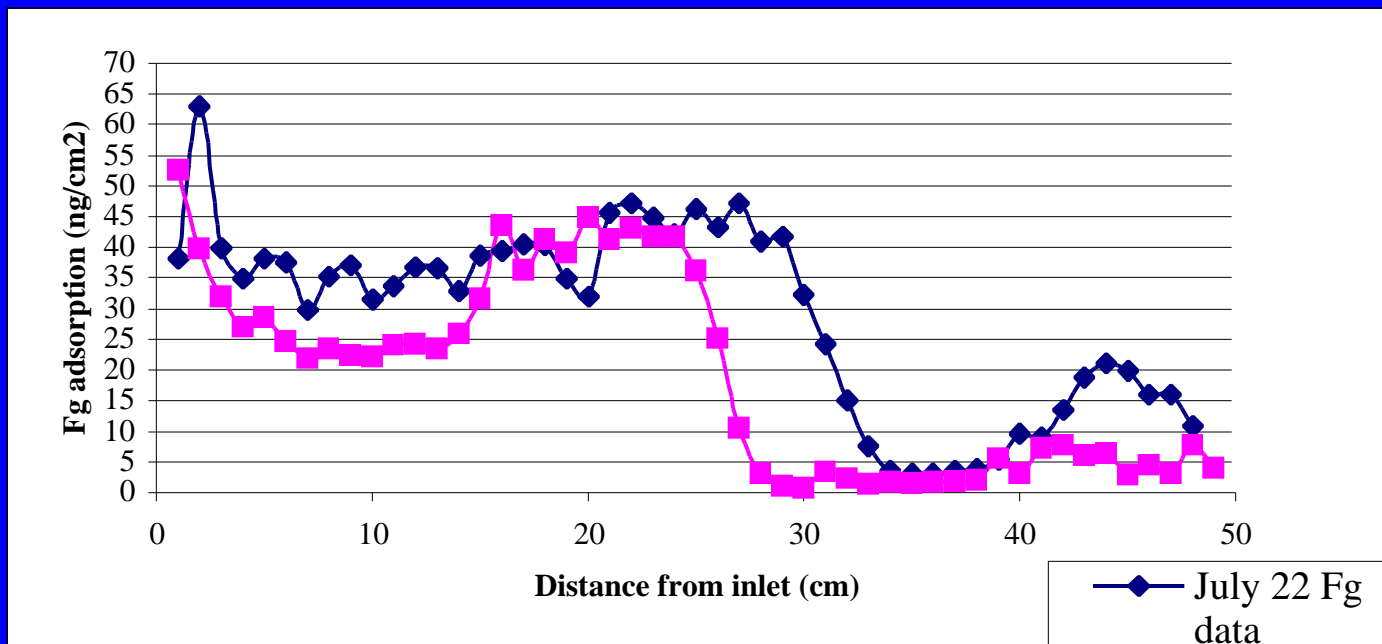


Ultralow fibrinogen adsorption biomaterials: Fg adsorption to plasma deposited tetraglyme on PE tubes Recent studies with modified conditions: Effect of argon addition

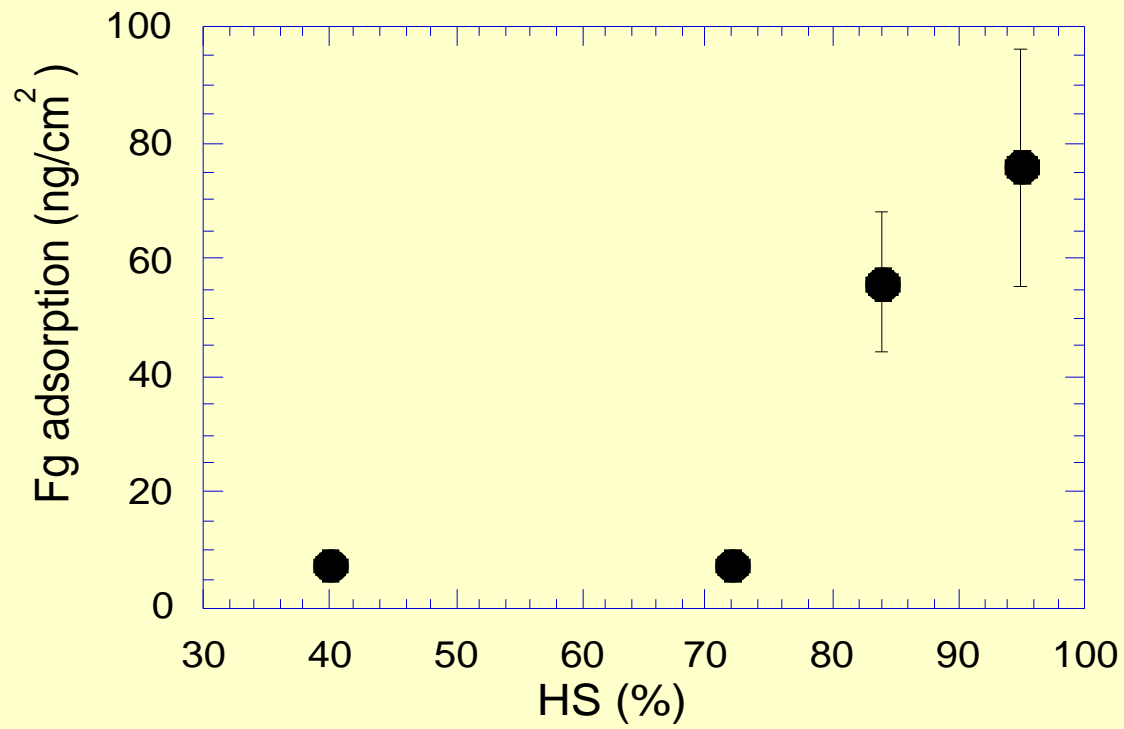
Reactor conditions: 150mT, 80W 1min, followed by 10 W for 10min.

Black curve: 0.8 sccm tetraglyme;

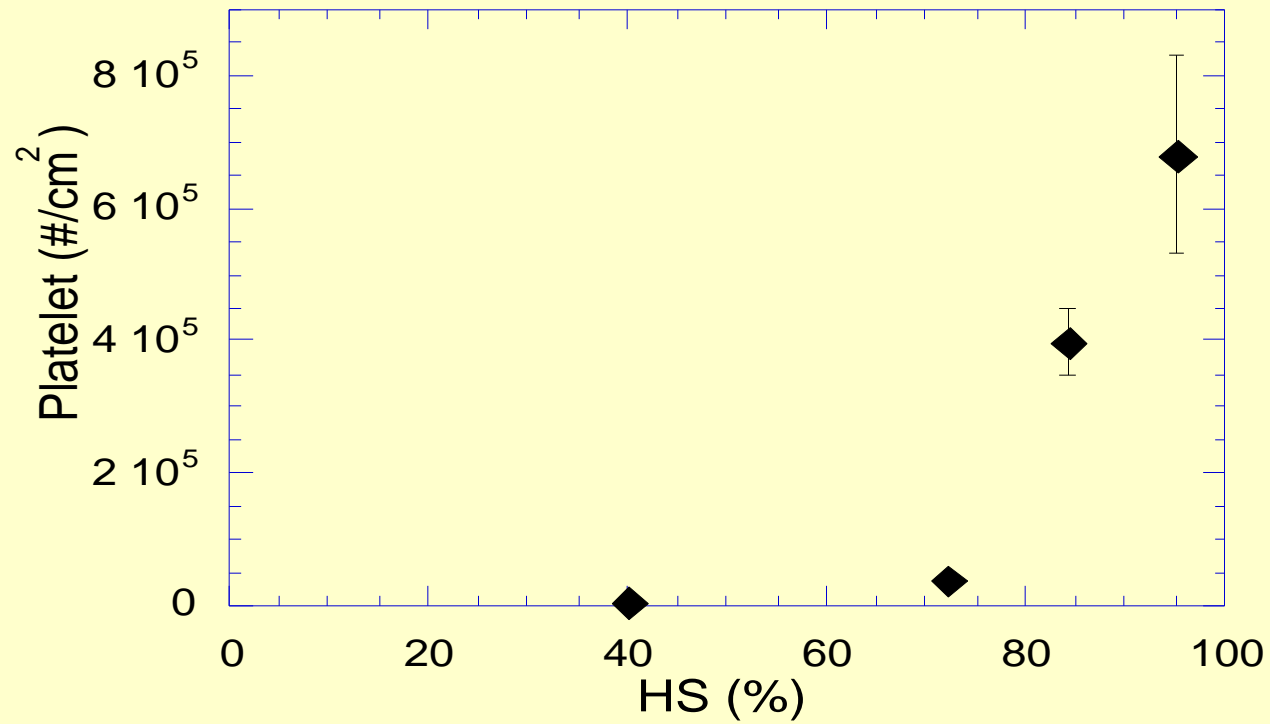
Pink curve : 0.5 sccm argon mixed with 0.8 sccm tetraglyme;



Fg adsorption from 1% plasma on PEG/PEU copolymer.



Platelet adhesion on PEG/PEU copolymer preadsorbed with 1% plasma.



Biological Activity of Adsorbed Proteins **And Biocompatibility: Conclusions**

- **The expression of the biologic activity of adsorbed adhesion proteins is the major recognition system that allows the body to react to foreign materials that themselves lack any intrinsic recognition motif.**
- **The biologic activity of adsorbed proteins is affected by both affinity and molecular potency.**
- **Lowering the affinity is a sound approach to making more biocompatible biomaterials, provided that ultralow affinity can be achieved.**