



Hydromer's New Non-Leaching Anti-Thrombogenic Coating F202

The Anti-Coagulative Properties of Heparin Bonded to Different Substrates

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Introduction

Heparin is one of the oldest drugs still in widespread clinical use and was originally isolated from liver cells. Heparin is a heterogeneous group of straight chain anionic mucopolysaccharides, called glycosaminoglycans containing different sugars joined by glycosidic linkages, forming polymers with molecular weights ranging from 6 KDa to 15 KDa. Heparin is strongly acidic because of its content of covalently linked sulfate and carboxylic groups. Under physiological conditions the ester and amide sulfate groups are deprotonated and attract positively charged counterions to form a heparin salt.

Blood coagulation or hemostasis is an important host defense mechanism. Heparin inhibits reactions that lead to the clotting of blood and the formation of the fibrin clot both *in-vitro* and *in-vivo* by acting at multiple sites in the normal coagulation system. Antithrombin III (ATIII) binds to a specific pentasaccharide sulphation sequence with the heparin polymer leading to a conformational change which results in its active site being exposed. When this occurs, thrombin binds forming a ternary complex and the inactivation of thrombin is increased 1000-fold.

Heparin is not only used as an injectable anticoagulant but is also used to form an inner anticoagulant surface on various experimental and medical devices. The purpose of this study was to determine how effective heparin would be as an anticoagulant once it is crosslinked and bound to various surfaces, such as polyurethane, polyvinyl chloride, silicone and stainless steel.

Methods

- Prior to all studies, surfaces were continuously washed with PBS until no free heparin could be detected in the supernatants.
- Citrate-treated whole blood was obtained from Biological Specialties, Inc. (Colmar, PA.). Clotting of whole blood was accomplished in two ways: either (1) One hundred (100) ul of whole blood was mixed with 100 ul Thromboplastin reagent or (2) equal volumes of whole blood and 0.020M CaCl₂ were mixed.
- A Fibrin Timer (American Labor, Durham, NC) was used to determine the Prothrombin Time in plasma according to the manufacturer's instructions (Pacific Hemostasis, Middletown, VA)).

Results

1. Polyurethane

(Figure 1) shows that no clot formation occurs when whole blood is incubated in the presence of Thromboplastin on the surface of coated polyurethane (PU) film. This is in contrast to what is observed on the control (uncoated) film. Heparin bonded to this surface appears very stable since these films have been stored for over 4 months @ RT in saline and still exhibit good anti-coagulative activity. Toluidine blue staining confirmed that heparin was bound (Figure 2). Polyurethane tubing was also coated and tested for anti-coagulative properties. The tubing shows no clot formation on the inside and the outside after exposure to calcified whole blood for 30 minutes @ RT (Figure 3).

Figure 1
(Polyurethane Film with 5 minute exposure to human blood)

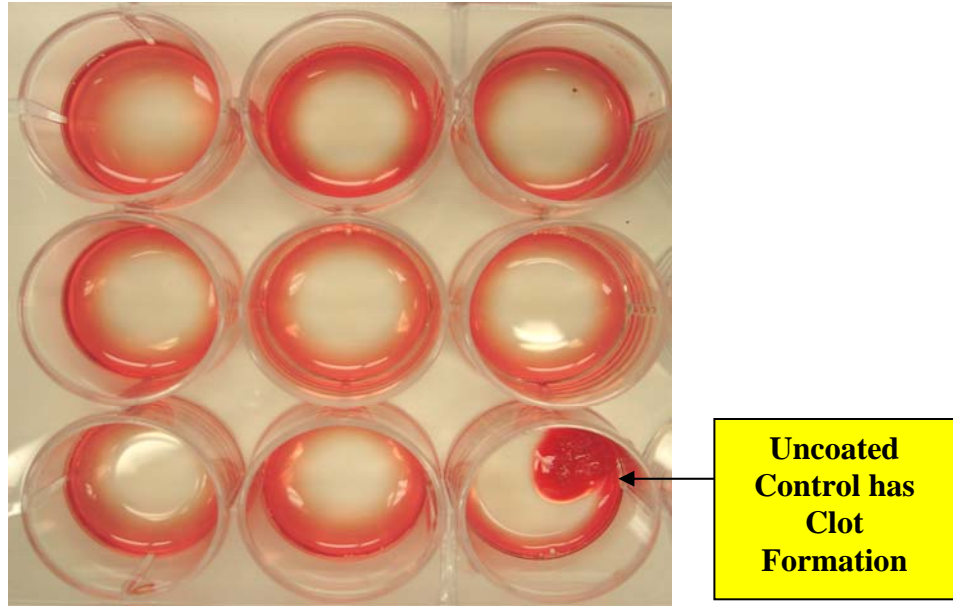
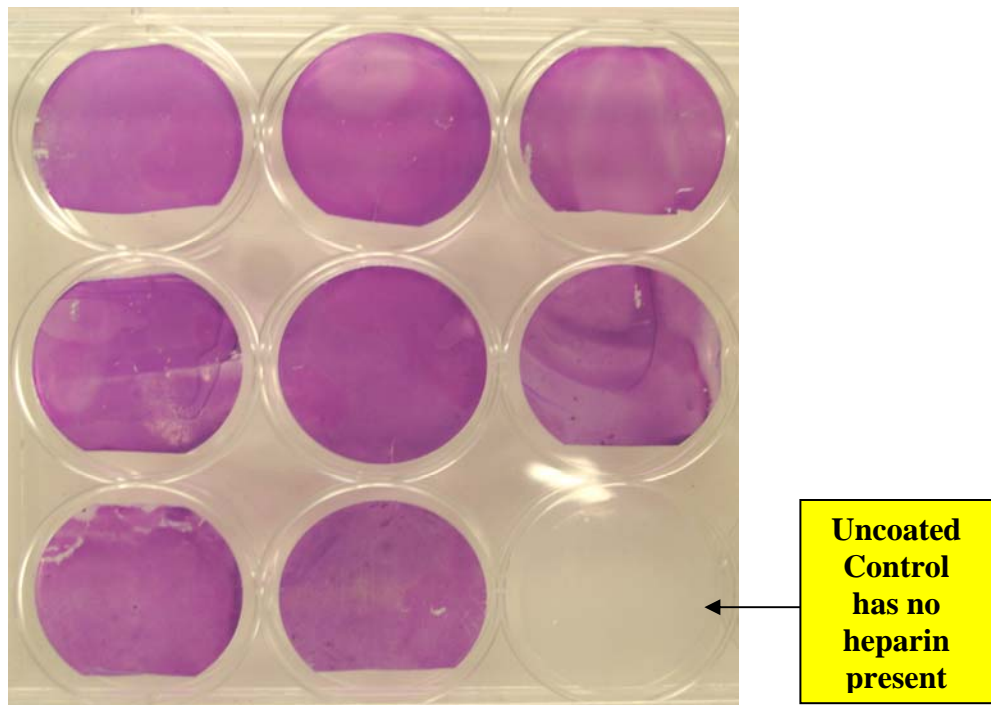


Figure 2
(Polyurethane Film with 5 minute exposure to human blood)
(Washed and then stained to confirm presence of bonded heparin)

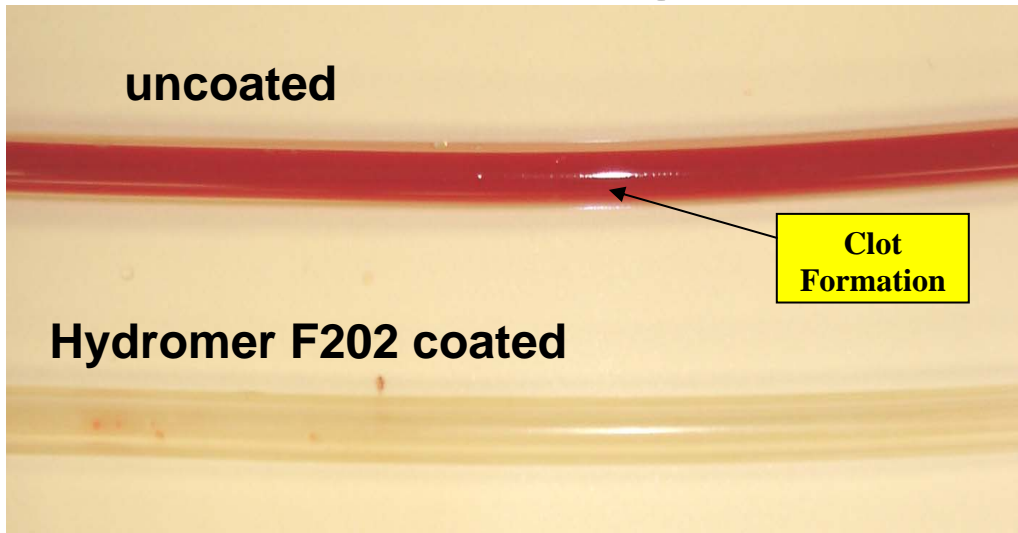


(Figure 3)

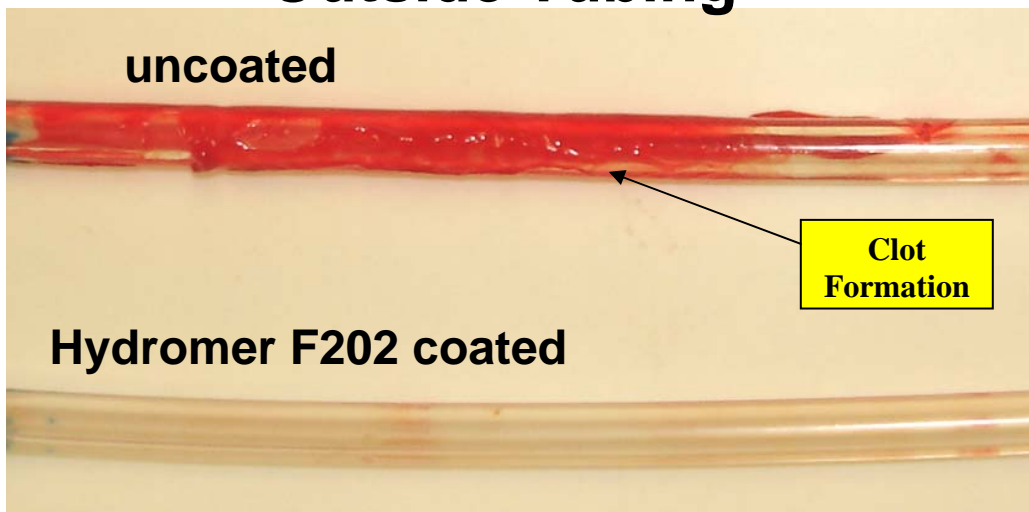
Polyurethane tubing

Exposed to human blood for 30 minutes

Inside Tubing



Outside Tubing



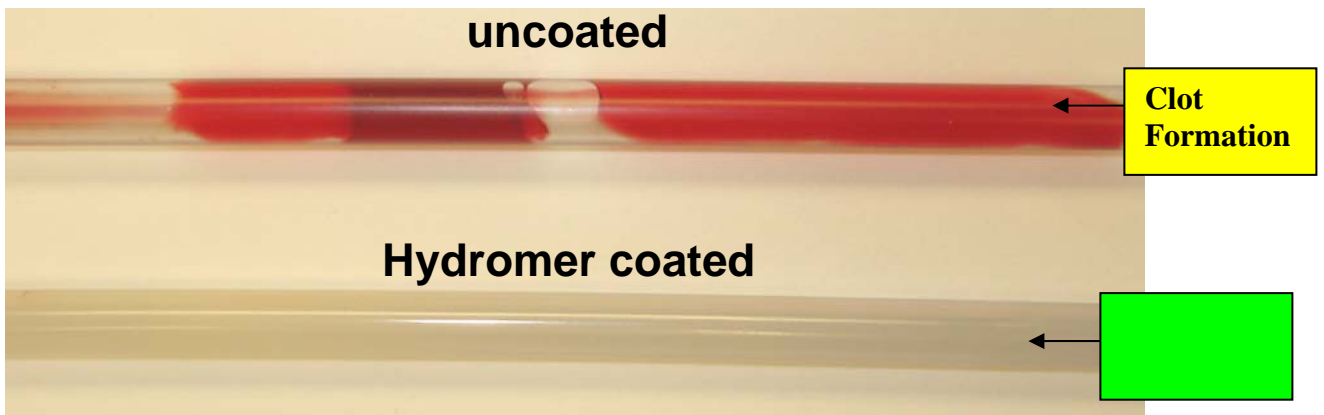
Polyvinyl Chloride (PVC) Tubing

Polyvinyl chloride was coated with our formulation and tested in a similar manner to PU. Figure 4 shows an excellent anti-coagulative effect on both the inside and the outside of the coated tubing.

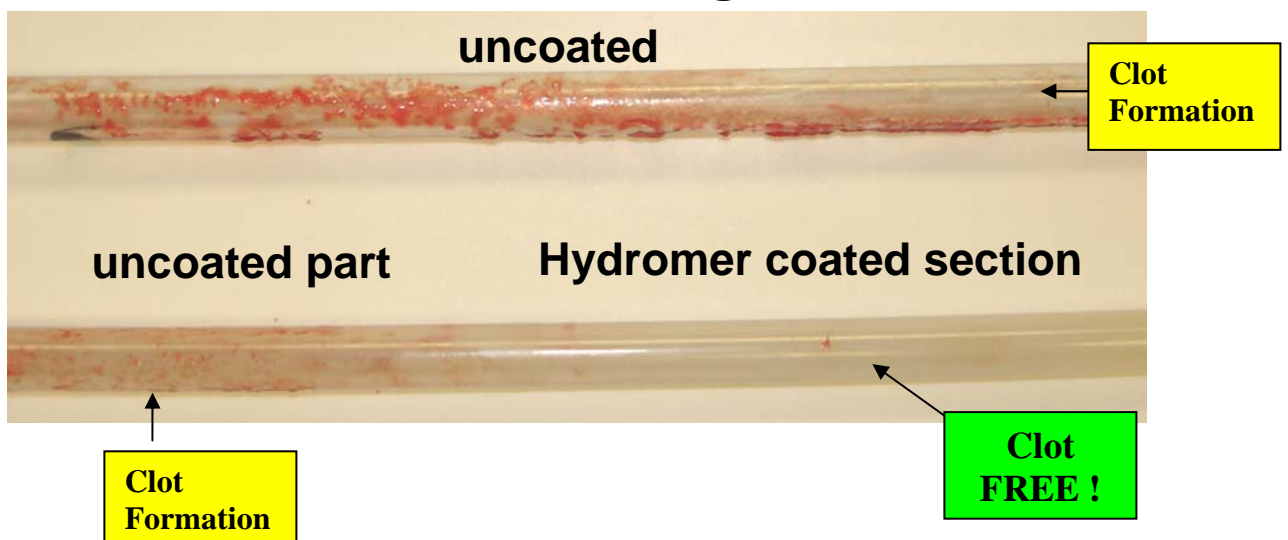
(Figure 4)

Exposed to human blood for 30 minutes

Inside Tubing



Outside Tubing

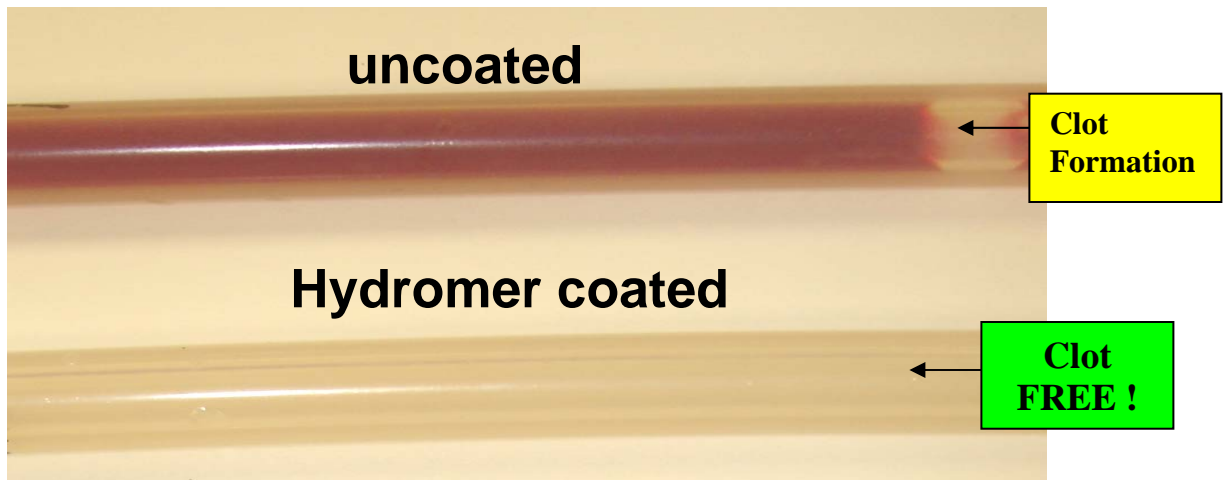


Silicone tubing

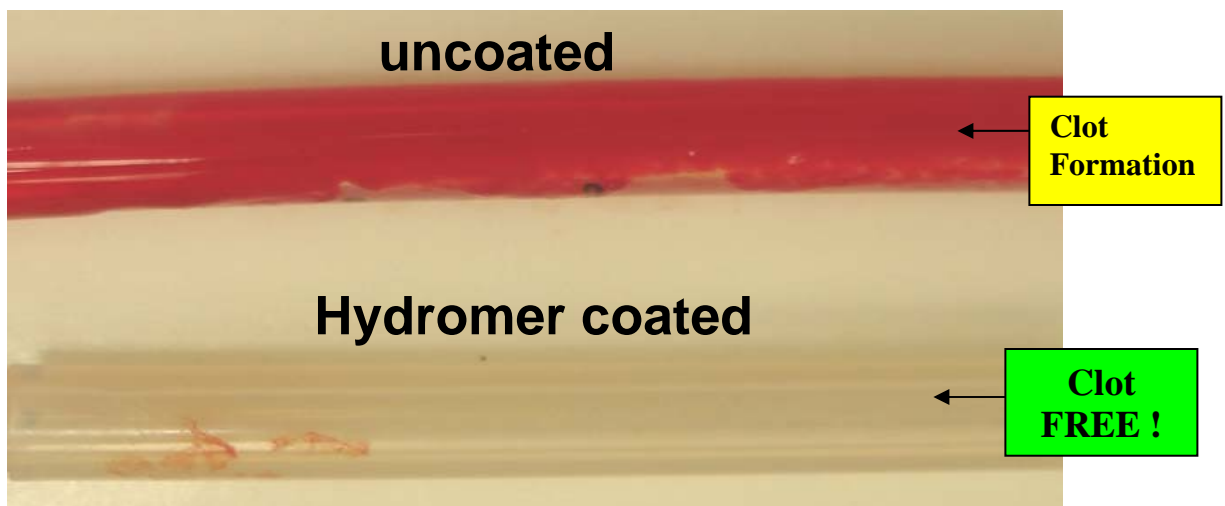
Tubing was tested in a similar manner to PU and PVC. Clot formation in the control tubing was prominent whereas the Hydromer coated tubing was free of clots.

(Figure 5) Exposed to human blood for 30 minutes

Inside Tubing



Outside Tubing



Stainless Steel Stents

Stainless steel was coated with our Hydromer formula F202 and tested for its ability to inhibit calcium-stimulated coagulation. The data below describes that there is no unbound heparin on the Hydromer coated surface. It can also be seen that when the coated stent was incubated in whole blood for 1 hour @ RT that whole blood and plasma failed to clot after the addition of Thromboplastin and that the plasma from that whole blood exhibited very high Prothrombin Times.

Leaching Test Each stainless steel stent was incubated in 2 ml of PBS for 12 hours and the PBS tested for Prothrombin Time.

	PT Time
	<u>(sec)</u>
Control	10.6
Bonded 1% Heparin	12.5
Bonded 3% Heparin	12.7

Plasma and Whole blood Each stainless steel stent was incubated for 1 hour @ RT in 2 ml citrated human whole blood. Then 100 ul of whole blood was mixed with 100 ul Thromboplastin and the time for clot formation recorded. In addition, 0.50 ml of this whole blood was centrifuged and the plasma was tested for Prothrombin Time and clotting.

	PT Time (Sec)	Whole Blood (Clot Test)	Plasma (from whole blood)
Control	16.9	Clot	Clot
Bonded 1% Heparin (H1)	>100	No Clot	No Clot
Bonded 3% Heparin (H3)	>100	No Clot	No Clot

Figure 6 (After 60 minutes exposure to blood)

Blood and Plasma Removed to Aid in the Visualization of Clot Formation

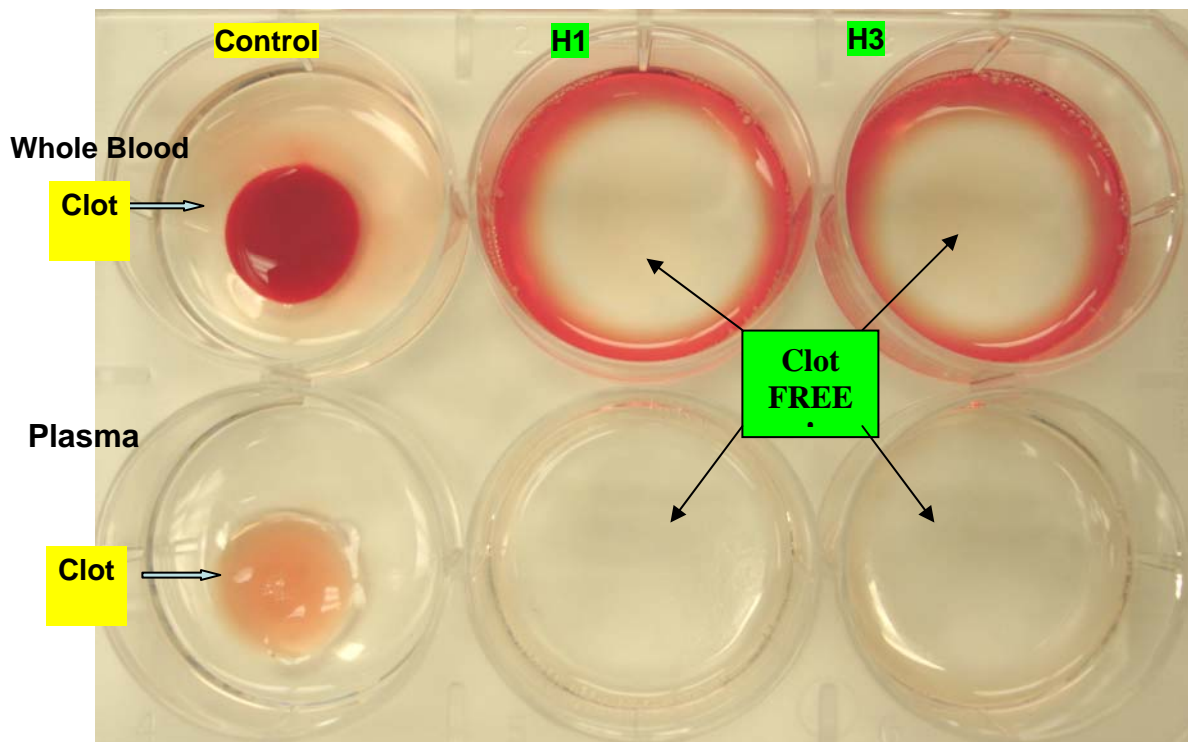


Figure 7

Heparin Staining By Toluidine Blue
After 60 minute exposure to human blood

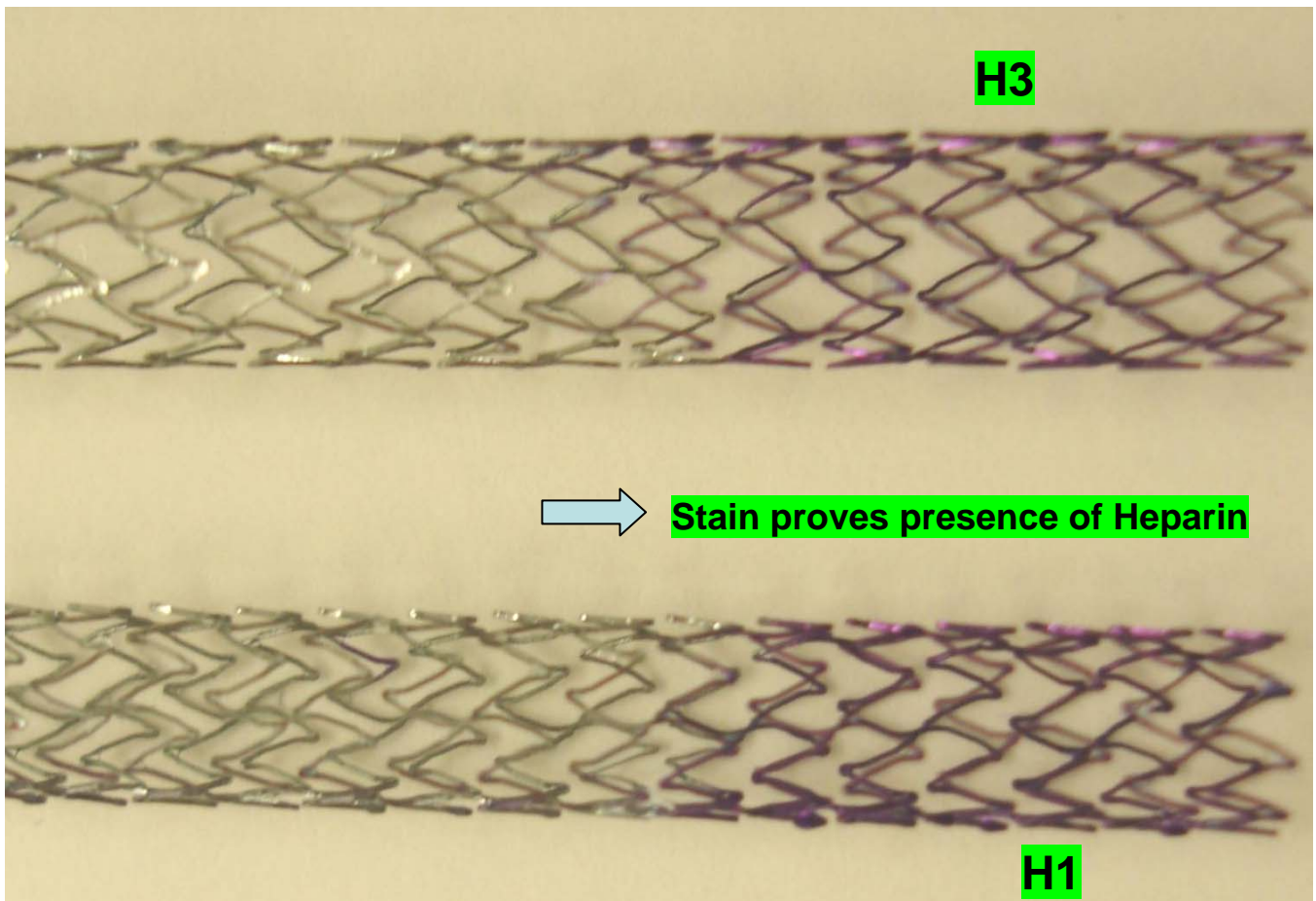
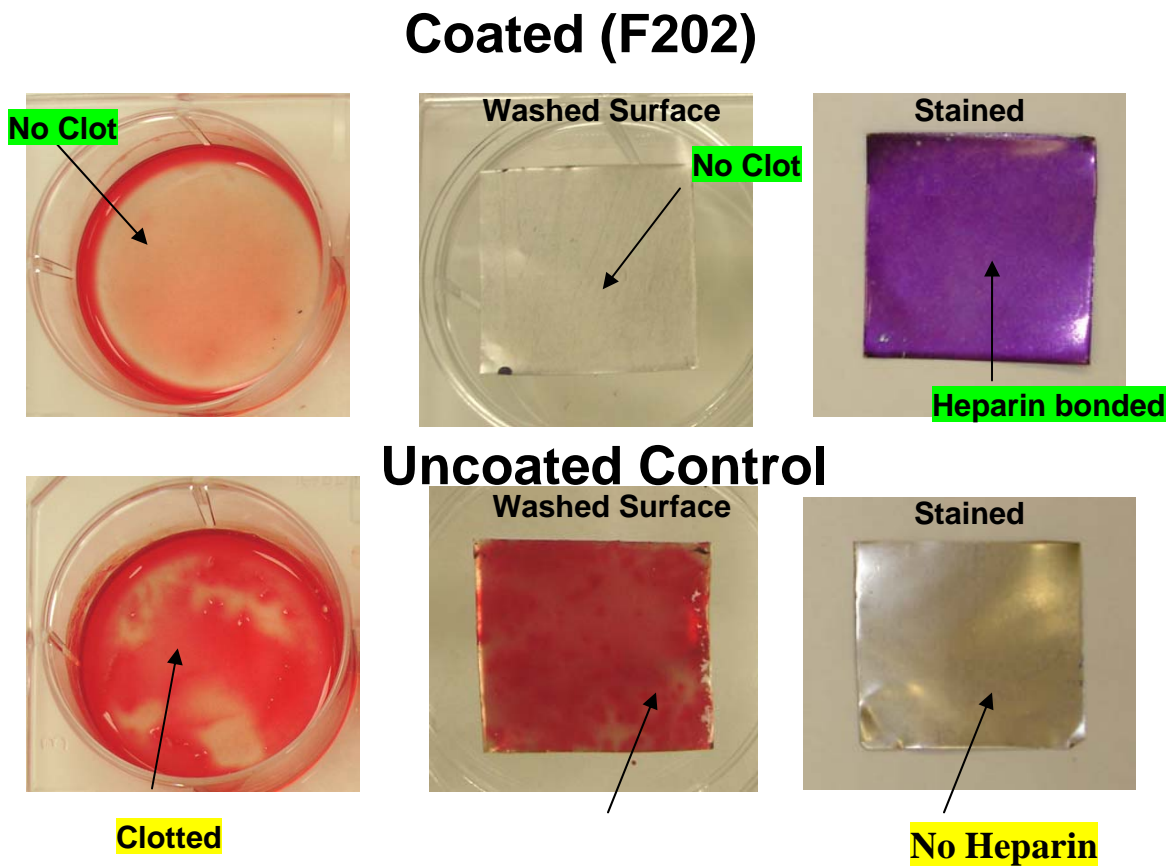


Figure 8

**After a 30 minute exposure to Human Blood,
Hydromer F202 Coated Stainless steel prevented clot formation
and stained positive with toluidine blue.**



Conclusion

Heparin is known to function as an anti-coagulant by interacting with anti-thrombin III and converting it to a more potent inhibitor of thrombin. We utilized simple qualitative tests in order to confirm that heparin not only was bound to the various surfaces but also that it was able to prevent CaCl₂-induced coagulation. Heparin also appears very stable when crosslinked to surfaces, in particular polyurethane since extensive washing with PBS did not alter its ability to prevent clotting. In conclusion, we have demonstrated that heparin retains long-lasting anticoagulative activity after bonding to a variety of coated solid supports.

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