

# Diagnosing Hemo(in)compatibility From Clinical and Device Considerations to Bench Assessment

## Hemo(in)compatibility – A Complex Puzzle

Medical device hemo(in)compatibility, which occurs when a blood-contacting medical device induces thrombus (blood clot) formation, can pose a significant threat to the health and safety of patients (Figure 1). Device-induced thrombi can be harmful and potentially life-threatening to patients if they cause device failure and clinical adverse events. If thrombi embolize (detach) from the surface of the device, critical blood vessels may be blocked, thereby restricting or interrupting blood flow and causing severe clinical complications, such as stroke or heart attack. Therefore, it is vitally important to diagnose and assess potential device hemo(in)compatibility to ensure efficacy before proceeding too far in the product development phase.

Proper diagnosis and assessment are often times akin to putting together an intricate puzzle due to the complex nature and multiple interacting factors involved in the coagulation process. To help device engineers and manufacturers evaluate and improve the hemocompatibility of their devices, we will discuss a systematic approach to piece together the hemo(in)compatibility “puzzle.” The following is a brief discussion of the mechanisms of blood coagulation, an enumeration of the critical factors that may contribute to device thrombosis, and an overview of several key bench-top tests that engineers and manufacturers can use to

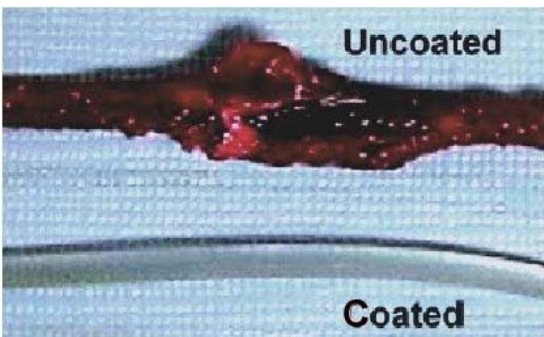


Figure 1. Thrombus formation on uncoated and heparin-coated polyurethane catheter materials following one hour exposure in a blood flow loop test.

## Mechanisms of Blood Coagulation

The human body has the innate ability to use blood coagulation to quickly arrest bleeding, a process known as hemostasis. In this case, blood coagulation is vital to prevent a small cut from turning into a life-threatening injury. To begin the clotting process, chemicals are released from the damaged blood vessel and trigger a complex chain of cellular and molecular reactions in

assess and quantify the potential hemo(in)compatibility of medical devices. A glossary of key terms is included as a reference.

the blood coagulation pathway (Figure 2). The several positive-feedback loops of the complex pathway illustrate how a trivial initial event can quickly amplify into a formed blood clot.

Blood is composed mainly of platelets, a variety of blood cells, salts, and normally inactive soluble proteins called clotting factors. Upon injury or exposure to a foreign object, the clotting factors become activated, and generate an enzyme called thrombin. Thrombin converts the soluble protein fibrinogen into insoluble fibrin. The formed fibrin acts as the foundation of the blood clot by providing a scaffold for the entrapment of platelets, blood cells, and plasma proteins. Once a fibrin clot is formed, the clot itself can promote more clotting. Whereas this process is crucial to the healing of an open wound, it becomes problematic when it occurs in connection with the use of a blood-contacting medical device.

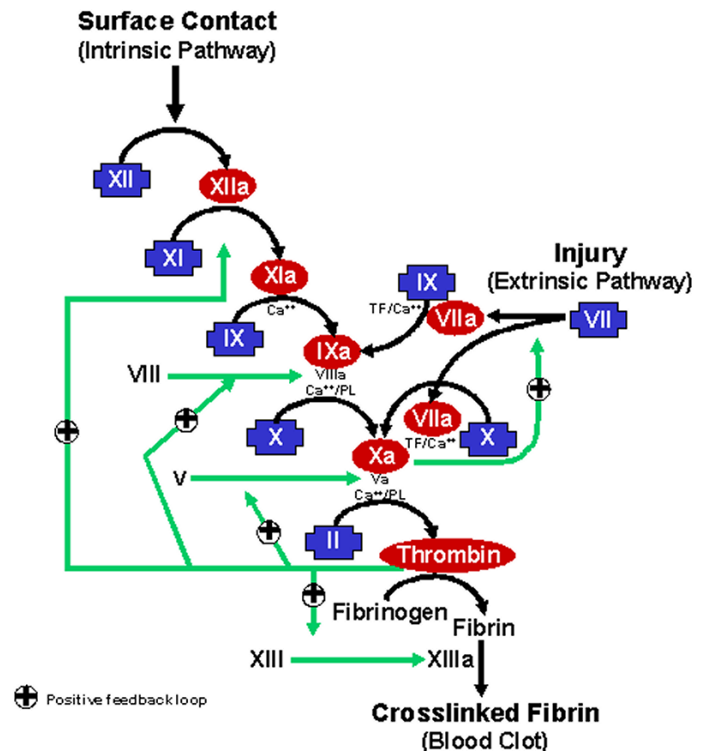


Figure 2. Abbreviated blood coagulation cascade showing both intrinsic and extrinsic pathways. The intrinsic pathway is the principal mechanism in causing device-induced thrombus formation.

When a medical device comes in contact with blood, several events can take place at the blood-device surface interface that can trigger the blood coagulation cascade (Figure 2). Within milliseconds of blood exposure, a layer of protein adsorbs to the device. Adsorbed Factor XII, an inactive protein in blood, becomes activated (Factor XIIa), and initiates the clotting cascade. Fibrinogen is required to form a protein meshwork of fibrin on the surface of the device. Fibrinogen also acts as an adhesive, causing platelets to attach to the surface of the device. Once platelets adhere to fibrinogen they become activated and release several chemical factors that cause other platelets in the vicinity to adhere to one another. This phenomenon is called “platelet aggregation.” The surface-aggregated platelets then encourage more fibrinogen-to-fibrin conversion by producing additional thrombin and thus fuel the coagulation cycle.

**EXAMPLES OF DEVICES THAT MAY HAVE HEMO/INCOMPATIBILITY ISSUES**

- Blood sensors
- Cardiac assist devices
- Central venous catheters
- Congestive heart failure devices
- Electrophysiology catheters
- Embolic protection devices
- Extracorporeal therapy devices
- Heart valves
- Hemodialysis catheters
- Neurological guidewires and catheters
- Septal defect repair devices
- Stimulation leads
- Thrombectomy devices
- Vascular grafts
- Vascular stents

Thrombogenic devices placed in slow-flow or stagnant systems, such as veins, could form what is known as “red thrombus,” which is composed of red cells with large amounts of fibrin. The stagnant condition prevents the flushing of activated clotting factors from the surface of the device, thereby producing a large amount of fibrin that entraps the red cells. Thrombogenic devices placed in high-flow systems, such as arteries, could form “white thrombus,” which is mainly composed of platelets held together by smaller amounts of fibrin. Under high-flow conditions, the amount of blood may be sufficient to dilute thrombin and other activated clotting factors, resulting in less fibrin formation. However, even with the high-flow situation, the flow velocity of blood at the vessel-wall surface is much slower than that at the center of the vessel lumen, and placement of a device into the vessel may cause turbulence immediately downstream from the device.

**Clinical and Device Hemo/Incompatibility Factors – Pieces of the Puzzle**

The mechanisms of device thrombosis are very complex. Each device can form thrombi by initiating a unique set of blood clotting responses. A device type that has performed well in one anatomical site may have problems at a different anatomical site. Based on years of research and observation, several clinical-situation and device-design factors have been identified that may influence the overall hemo/compatibility of a device, alone or in combinations. Among these are: (1) blood flow dynamics (flow rates and potential turbulence), (2) device geometry, (3) the duration of blood contact, (4) surface area, (5) the type of material used to fabricate the device, and (6) surface roughness (Figure 3). Device engineers need to evaluate and consider each factor and potential interactions among the various factors when developing a blood-contacting medical device, and diagnose any potential hemo/compatibility issues in the early stage of product development.

Blood Flow Dynamics

Blood flow controls the rate of the transport of blood cells and proteins to the surface of a medical device, and can greatly affect the formation and composition of device-induced thrombi.

Device Geometry

Device geometry dramatically affects the dynamics of blood flow at or near the device surface. Disturbance of laminar blood flow by a device can produce turbulence that induces thrombus formation. In areas of turbulence, platelets can be forced to the surface of the device causing activation and aggregation. Turbulence can also produce stagnant regions of blood at particular sites of the device that allow activated blood factors to accumulate and, thus, facilitate the formation of thrombi. In general, the more complex the device geometry, such as an artificial heart, the greater the chance of turbulence and potential hemo/compatibility.

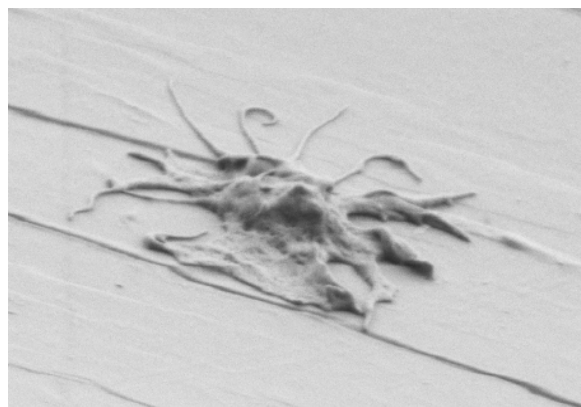


Figure 4. SEM image of platelet beginning to spread on an uncoated polyurethane surface.

Duration of Blood Contact and Surface Area

Blood responses to a medical device can also vary depending on the length of time the device surface is in contact with blood. The primary concern for short-term devices, those exposed to blood for minutes to hours, is the formation of significant thrombi that could interfere with the device’s function, and the embolization of formed thrombi during manipulation of the device, resulting in vessel occlusion downstream. Long-term devices, such as heart valves, central venous access catheters, and vascular grafts, may be exposed to blood for days, months, or

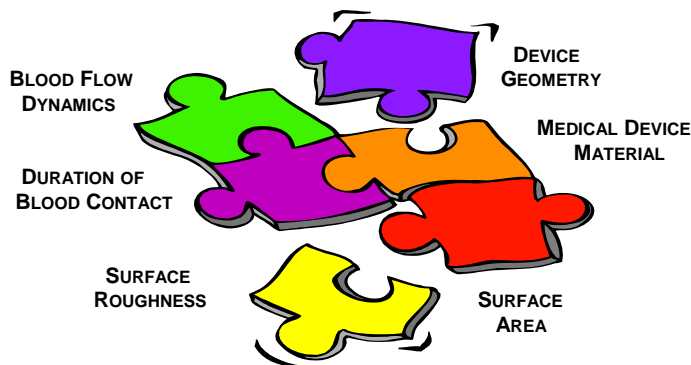


Figure 3. Clinical and device hemo/compatibility factors—pieces of the puzzle.

years. The long-term use of these devices may produce profound systemic effects such as anemia or may promote infection and calcification.

Devices that have large surface areas may cause the destruction or “consumption” of circulating blood cells and proteins near the surface of the device, thereby lowering their concentration in blood. When too many red blood cells are destroyed, conditions such as anemia can occur. Other complications could arise, such as clotting deficiencies, caused by the removal of platelets.

### Medical Device Material – Surface Chemistry and Roughness

Materials used to fabricate medical devices can possess different surface chemistries that can influence how aggressively blood elements respond to the surface of the device. For example, the surface charge (anionic or cationic) can influence plasma protein adsorption on the device surface. Currently, there is little consensus as to which materials are blood compatible, although anionic heparin coating often improves device performance (Figure 1). In general, most synthetic polymers and metal substrates are thrombogenic; the human biological defense system views them as foreign materials.

Furthermore, it is not always the case that if the materials comprising a device are blood compatible, a device fabricated from those materials will also be blood compatible due to other contributing factors, such as complex device geometry.

Besides surface composition and chemistry, the degree of surface roughness can also play a significant role in the hemo-

compatibility of a device. A rough surface has a higher affinity for platelet attachment (Figure 4) and may cause micro-turbulence at the surface of the device. It should be noted that most surfaces are rough at the microscopic level even if they appear smooth to the naked eye. In general, researchers believe that microscopically smooth surfaces, and more intriguingly, surfaces that have been micro-textured with a precise specificity, are more hemocompatible than surfaces with irregular, uncontrolled roughness.

### Bench-top Tests for Assessing Hemoincompatibility

The initial diagnosis of the relative hemocompatibility or hemoincompatibility of a device is assessed by consideration of the critical clinical and device hemoincompatibility factors mentioned above. There are also several bench-top tests (in vitro) or animal models (in vivo or ex vivo) that can be used to measure the degree of hemoincompatibility of medical devices. Several in vitro test methods can be employed to obtain quantifiable measurements. Descriptions of four key tests, fibrinogen adsorption, contact activation, platelet adhesion, and

blood flow-loop, are summarized in Table 1. Figure 5 shows the results of a static platelet adhesion test on a thrombogenic surface compared to a hemocompatible surface. In this test, human platelets in plasma were incubated on test samples for one hour. Samples were then washed to remove any loosely adhered platelets. The platelets were detected using a platelet-specific fluorescent probe, and images were acquired with a microscope and digital camera. From the images, percent platelet coverage and platelet morphology can be determined. An in vitro blood loop, as shown in Figure 6, can be utilized to test the relative thrombogenic potential of surfaces under flowing whole blood. Sur-

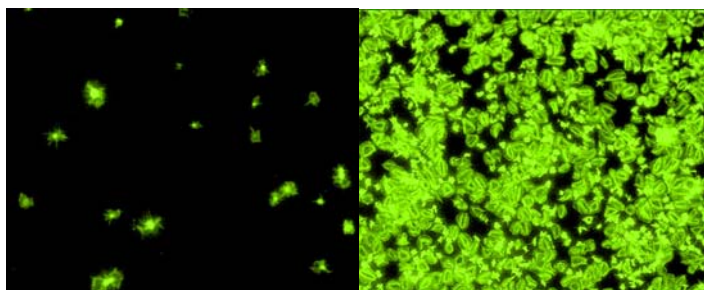


Figure 5. Fluorescent microscopy images showing results from a platelet adhesion assay—a hemocompatible surface (left) vs. a thrombogenic surface (right).

Table 1. In vitro hemocompatibility assessment methods. The examples shown were selected to represent some key tests from a variety of testing options that can be utilized.

BLOOD-MATERIAL INTERACTION	ASSESSMENT METHOD	DESCRIPTION	TESTING RATIONALE
Protein Adsorption	Radiolabeled Fibrinogen	Samples are incubated in a radiolabeled fibrinogen solution, washed, and counted for radioactivity.	Fibrinogen adsorption is necessary for surface-induced fibrin formation and promoting platelet attachment.
Contact Activation	Factor XIIa Assay	Samples are assayed for Factor XIIa by spectrophotometric methods and a Factor XIIa-specific chromogenic substrate.	Upon adsorption to a surface, Factor XII becomes activated (Factor XIIa) and initiates the coagulation cascade.
Platelet Adhesion	Fluorescence Microscopy	Platelets are visualized using a fluorescent probe, and percent platelet coverage is measured. Platelet activation is qualitatively assessed based on the morphology of the adhered platelets.	Platelets are highly sensitive to foreign surfaces and can contribute to blood coagulation by releasing several procoagulant factors.
Flowing Blood-Material Interactions	Blood Flow Loop	Fresh bovine blood containing radiolabeled platelets is circulated through a flow loop at 37°C. Samples are visually inspected for thrombus formation. Platelet adhesion is assessed by detecting radiolabeled platelets. FACS, RIA, and ELISA techniques can be used to monitor platelet activation.	Complex device geometry can affect the dynamics of blood flow, making a device more prone to thrombus formation.

faces can be visually inspected and, if so desired, thrombus can be quantified by measuring for radiolabeled platelets. It is important to note that each test provides only one piece of the hemoincompatibility puzzle, and a variety of tests must be run to complete the puzzle and get a clear picture.

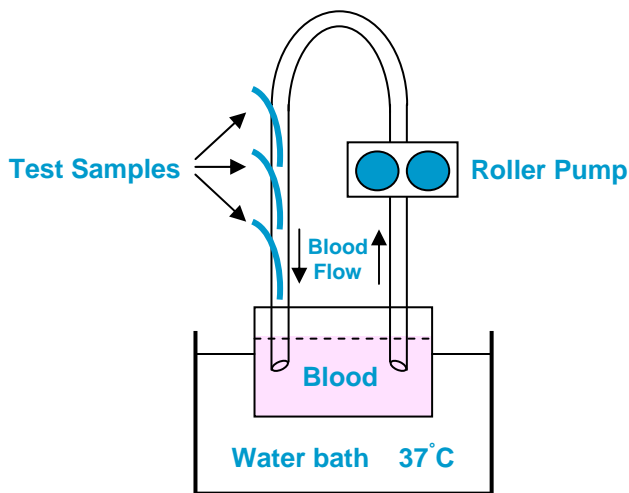


Figure 6. Schematic of the recirculation bovine blood *in vitro* model.

## Conclusion

In summary, we have provided a framework for device engineers to evaluate and examine various pieces of the hemoincompatibility puzzle and then assemble those pieces to solve the puzzle. We have presented the following steps for diagnosing the hemoincompatibility of your device:

1. Review the blood coagulation pathway to appreciate the different mechanisms that can initiate the clotting process.
2. Evaluate how the various clinical and device hemoincompatibility factors may affect the performance of your device, and determine how these factors may interact with one another to produce a compounding effect.
3. Utilize some of the various relevant bench-top assays to quantify the degree of hemocompatibility or hemoincompatibility of your device. Further, it should be noted that *in vitro* hemocompatibility does not guarantee *in vivo* or clinical device hemocompatibility which can only be evaluated in pre-clinical animal models and human clinical trials.

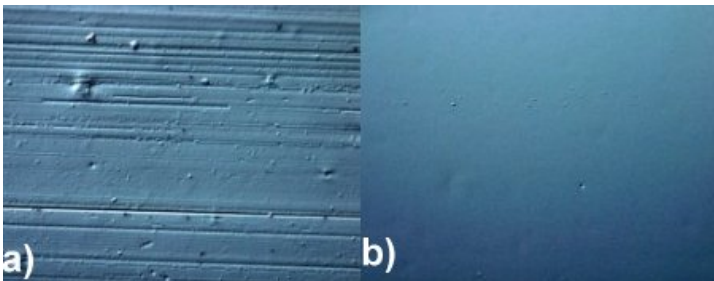


Figure 7. (a) Microscopic visualization of a seemingly smooth surface shows many rough areas that could promote thrombus formation. (b) The same surface with a passivating hydrogel coating smoothes the surface, and reduces protein adsorption and platelet attachment.

If a potential hemoincompatibility problem is indicated, device engineers must consider the next challenging puzzle – how to rectify the problem and improve the hemocompatibility of the device. As the device hemoincompatibility problem is largely a surface phenomenon, one effective approach is to utilize surface

modification technology to improve the hemocompatibility of the device surface without affecting the other physical properties or dimensions of the device. One example of how a surface coating can improve the hemocompatibility of a device is shown in Figure 7. SurModics' companion white paper, "Advancements in Blood-Compatible Coatings," provides an overview of various hemocompatible surface modification solutions that are the culmination of over 15 years of research and development. SurModics is a pioneer and the leader in providing comprehensive surface modification solutions to medical device manufacturers.

For questions regarding hemoincompatibility issues, from diagnosis of the problem to surface modification solutions, please contact SurModics at 952-829-2700, toll free at 866-SURMODX, or email us at [info@surmodics.com](mailto:info@surmodics.com).

## GLOSSARY

**Emboli** – Dislodged thrombus.

**Enzyme** – A protein that increases the rate of (catalyzes) a biochemical reaction.

**Factor XII** – (Hageman Factor) a glycoprotein found in blood plasma.

**Factor XIIa** – An enzyme (the activated form of Factor XII) which is central to the initiation of the intrinsic coagulation pathway.

**Fibrin** – The insoluble protein that forms fibers and is the foundation of a blood clot.

**Fibrinogen** – A soluble blood protein necessary for fibrin formation.

**Platelets** – Small disk-shaped cells that are abundant in blood and contribute to the promotion of blood coagulation. Platelets are highly sensitive to foreign surfaces.

**Thrombin** – The enzyme that catalyzes the formation of the insoluble protein, fibrin, from soluble fibrinogen.

**Thrombogenic** – Clot promoting.

**Thrombus, thrombi** – Blood clot(s) -- the aggregate of a network of fibrin, platelets, and other blood components.

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