Preclinical Device Thrombogenicity Assessments: Key Messages From the 2018 FDA, Industry, and Academia Forum

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Device-related thrombosis and thromboembolic complications remain a major clinical concern and often impact patient morbidity and mortality. Thus, improved preclinical thrombogenicity assessment methods that better predict clinical outcomes and enhance patient safety are needed. However, there are several challenges and limitations associated with developing and performing preclinical thrombogenicity assessments on the bench and in animals (*e.g.*, the clinical relevance of most *in vitro* tests has not been established, animal studies may not accurately predict clinical thrombotic events). To facilitate a discussion on how to overcome some of these challenges and to promote collaboration between the Food and Drug Administration (FDA), industry, and academia for the development of more reliable test methods, a scientific forum was organized by FDA and held in Washington, DC, on June 15, 2018 at the ASAIO 64th Annual Conference. Three subject matter experts from the medical device industry and FDA presented their perspectives at this forum, and several audience experts provided input during the open dialogue session. This article summarizes the key messages from the forum regarding the current status and challenges of preclinical thrombogenicity testing, important areas of needed research, and mechanisms for working with FDA to further improve thrombogenicity evaluations of medical devices. *ASAIO Journal* 2021; 67:214–219.

Key Words: *in vitro* testing, animal testing, blood-contacting devices, biomaterials, leukocyte activation, platelet activation, thrombosis, thrombogenicity

Blood-contacting medical devices such as catheters, stents, artificial heart valves, oxygenators, and ventricular assist devices (VADs) have been used to treat cardiovascular disease in millions of patients. However, device-related thrombosis and thromboembolic complications remain a major clinical concern and often contribute substantially to patient morbidity and mortality. Thus, improved preclinical thrombogenicity assessment methods that better predict clinical outcomes and enhance patient safety are needed.[1–8]

There are several challenges and limitations associated with preclinical thrombogenicity assessments. Animal implant models are commonly used to demonstrate that a device is reasonably safe before patient use, particularly for long-term implants such as VADs. However, the inherent differences in blood properties and anatomy between species make selecting and designing the appropriate animal model challenging and limit the clinical relevance of animal study results.[3,9–12] In addition, these *in vivo* tests may be burdensome and often require the animals to be euthanized for pathological examination of thromboembolic events.

Many *in vitro* hemocompatibility assays have been developed to evaluate device thrombogenicity and, in some cases, can be used to supplement, reduce, or replace animal studies.[13–18] These bench-top assays are usually less burdensome than animal studies, and many of these tests can be performed with human blood to address species differences. However, the clinical relevance of most *in vitro* tests has not been established. Additionally, *in vitro* testing alone is generally insufficient for evaluating chronic thrombogenicity of long-term implants caused by the nature of short-term bench-top testing. To facilitate a discussion on how to overcome some of the challenges associated with preclinical thrombogenicity assessments and to promote collaboration between the FDA, industry, and academia for the development of more reliable test methods, a scientific forum was organized by FDA and held in Washington, DC, on June 15, 2018 at the ASAIO 64th Annual Conference. Dr. Trevor Snyder, at the time with VADovations (Oklahoma City, OK), and now with CorWave (Clichy, France), Dr. Ina Laura Perkins from Scandinavian Real Heart AB (Västerås, Sweden), and Dr. Qijin Lu from the FDA (Silver Spring, MD) presented their perspectives at this forum. Several audience experts also provided input during the open dialogue session, moderated by Dr. Megan Jamiolkowski (FDA, Silver Spring, MD). Below is a summary of the forum proceedings. Please note that this is not a comprehensive review of preclinical thrombogenicity testing, but rather a discussion of the key messages from this forum.

Regulatory Aspects of Preclinical Thrombogenicity Testing

As discussed in an FDA Guidance Document on biocompatibility evaluation of medical devices, preclinical thrombogenicity evaluations of blood-contacting devices are essential to ensure patient safety and are generally needed for the regulatory approval/clearance of new medical devices.[19] Thrombogenicity evaluations may also be necessary when certain modifications to currently marketed devices are made. Typically, these modifications include changes that impact the physical and chemical properties of blood-contacting surfaces

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or the blood flow patterns through or around the devices. In addition, thrombogenicity evaluations are often performed at multiple stages during product development to screen biomaterials and device geometries for their hemocompatibility. The type of thrombogenicity testing that should be performed depends on many factors including implant location, blood contact duration, complexity of the device geometry, materials used, manufacturing and sterilization processes, and whether the device will be used with anticoagulation. A principal standard for evaluating blood-contacting medical devices, ISO standard 10993-4 (2017), was recently updated and serves as a useful resource for selecting potential tests; however, the specific test methods and protocols still need to be determined by the device developer.[13]

In vivo Testing

Preclinical animal implant studies are commonly used to demonstrate device safety and can range from acute implant tests, such as a nonanticoagulated venous implant (NAVI) study, to chronic safety or functional studies.[13,19] Multiple animal species have been used to evaluate device thrombogenicity. The most commonly used animal models are swine, ovine, canine, and bovine.[10,11] The type of animal model used may be limited by the size of the device, the growth rate of the animal, and the availability of the species.[13]

Animal studies can simulate many aspects of clinical use and provide valuable information on device safety and performance. They permit detailed measures of device function, blood-device interaction, and frequent in-life veterinary examinations of animals. They also allow the opportunity to conduct detailed pathological evaluations following chronic use of a device, which cannot be done in a clinical study. Despite these benefits, animal studies do not always accurately predict device thrombogenicity in humans. For example, some of the animal studies conducted on the HeartMate II LVAD (Abbott, Chicago, IL) and the HeartWare HVAD (Medtronic, Dublin, Ireland) reported minimal pump thrombosis.[20,21] However, a substantial number of thrombosis-related adverse events occurred during clinical use (*e.g.*, it has been reported that pump thrombosis and stroke events ranged from 7.5% to 11.3% and 10% to 44% at 1 year of VAD support, respectively).[6–8] Therefore, animal implant studies need to be improved and/or alternative test methods need to be developed to provide a better prediction of clinical outcomes in humans. Future considerations may include developing more feasible animal models of heart disease or heart failure and defining acceptable worst-case clinical use conditions for testing certain devices (*e.g.* VADs).

In vitro Testing

In some cases, for devices with short blood-contacting durations or when minor modifications are made to a long-term implant, a panel of *in vitro* assays that evaluate different contributing factors to thrombosis (*e.g.*, plasma coagulation proteins, platelet activation or adherence), along with assessment of surface geometry, may be appropriate in lieu of animal testing to address thrombogenicity risks.[19] There are two types of in *in vitro* tests for evaluating thrombogenicity: (1) static tests for examining material-mediated thrombogenicity and (2) dynamic tests for evaluating flow-mediated thrombogenicity. Compared with dynamic tests, static tests are easier to perform, and the test results tend to be more consistent. However, because these tests are performed in the absence of simulated clinical flow conditions, they can only be used to characterize device thrombogenicity related to the chemical properties of the blood-contacting device materials and are not useful in assessing device thrombogenicity related to blood flow and surface geometry. Many potential static test methods are listed in the FDA recognized ISO 10993-4 standard. However, this standard does not include any detailed test protocols..[13] Currently, there are only two complete test methods that have been standardized and subsequently recognized by the FDA: (1) ASTM F2382-18: Test Method for Partial Thromboplastin Time (PTT)[14] and (2) ASTM F2888-19: Test Method for Platelet/Leukocyte Counts.[15] Although these two assays are sensitive enough to differentiate between a very thrombogenic material (*e.g.*, positive control glass) and a thromboresistant one (*e.g.*, negative control polypropylene), they may not be able to distinguish subtler thrombogenicity differences among commonly used biomaterials. Although the aforementioned static thrombogenicity methods may be helpful in supporting a regulatory submission when appropriate, test methodologies with greater test sensitivity are needed to further enhance thrombogenicity assessments, along with standardized methods for handling blood samples and verifying blood reactivity before the testing. Moreover, to characterize the effects of device geometry and blood flow path conditions on the thrombotic potential of a device, dynamic assessments performed under clinically relevant flow conditions are also needed. Currently, there are no standardized or widely accepted *in vitro* test methods to evaluate dynamic device thrombogenicity, as the test methodology used in existing flow loop systems differ greatly.[16–18] Of note, while the clinical relevance of current *in vitro* testing is not well-established, these tests still provide useful device characterization information.

Developing New Preclinical Thrombogenicity Assessment Methods

Since device thrombosis is a complex issue that involves many time-varying factors, there are several key issues that need to be considered when developing new thrombogenicity test methods. Similar to the importance of designing an appropriate animal study that is tailored to the device under investigation, choosing appropriate blood parameters (species, anticoagulation, and collection methods) for testing a specific device use is also critical for *in vitro* thrombogenicity studies. For instance, Goodman identified significant differences in platelet adhesion of human, porcine, and ovine platelets onto model cardiovascular biomaterials.[10] Although using human blood can overcome species differences and is preferred for *in vitro* studies, using animal blood may be necessary for *in vitro* tests where a large blood volume is needed and where using human blood would not be feasible or practical. Moreover, handling human blood may also involve additional personnel, safety, and regulatory requirements that cannot always be met by medical device developers.

It has long been recognized that selecting the appropriate animal blood species is challenging because blood sensitivity can be significantly affected by test conditions. An examination of platelet adhesion to biomaterials under dynamic flow

identified substantial differences between human, baboon, macaque, canine, bovine, ovine, and porcine blood.[12] However, no universal agreement exists regarding the relative activity of blood from different species. Lu *et al*.[22] exposed bovine blood to varying shear stresses for 2 minutes using a cone-and-plate rheometer model and observed less platelet activation compared with sheared human blood when evaluated by three different platelet activation markers (*i.e.*, platelet count, plasma serotonin, platelet surface CD62P). In contrast, Chan *et al*. reported that sheared bovine blood had greater platelet activation than human blood using a CD62P platelet surface activation marker by flow cytometry, when the blood was similarly exposed to various shear rates using a rheometer, but for a longer shear exposure duration of 15 minutes.[11] Of note, one major limitation of the above studies was that the blood was continuously exposed to high shear stresses in the rheometers for 2 or 15 minutes in the *in vitro* tests, whereas blood passes through the high shear stress regions of a medical device (*e.g.*, heart valve, vascular graft, or VAD) in a period of milliseconds during a blood recirculation cycle.

Another important variable to consider is the type and concentration of anticoagulant used in an *in vitro* thrombogenicity test, as it can substantially affect the sensitivity of the assay. For example, the initial version of the ASTM F2888 (2013) standard for screening materials based on platelet and leukocyte counts was not recognized by the FDA, primarily because subsequent testing revealed that the use of sodium citrate anticoagulation could substantially mask the thrombogenicity response of different materials. Studies performed by the FDA and others[23,24] have shown that the sensitivity of this assay can be improved by utilizing a low concentration of heparin (approximately 1.0 U/ml) or by recalcifying the citrated blood and adding heparin anticoagulant (1.5–2.0 U/ml). This highlights the need for evaluating and selecting an appropriate anticoagulation strategy when developing new preclinical thrombogenicity test methods.

Another important consideration for developing *in vitro* test methods is determining what markers should be used to evaluate device thrombogenicity. When selecting the appropriate markers, it is important to consider the goal of the assessment. For some applications, markers such as visualizing thrombus formation or measuring the adherent thrombus weight may be sufficient for addressing the thrombogenicity potential. However, if the goal of the assessment is to optimize a design feature or improve material selection, more sensitive biomarkers such as thrombin–antithrombin (TAT) complex, thrombin generation, and platelet activation may be needed. Discovering and utilizing nontraditional thrombogenicity markers may also aid in producing more clinically relevant results. For example, preclinical and clinical evidence has shown that white blood cells are affected by blood-contacting devices such as VADs,[25–28] suggesting that evaluating white blood cell damage may provide useful insight into device thrombosis and may afford more clinically relevent predictions of safety.

Developing new *in vitro* screening tools to assist in the material selection for blood-contacting devices may reduce the time and cost of developing safe medical devices. For example, during his forum presentation, Dr. Snyder described tests employed by VADovations, in which material samples were rocked in recalcified human whole blood for 2 hours and then assessed by measuring different thrombogenicity markers. The thrombotic potential of the materials were then classified based on the time to peak thrombin generation, magnitude of peak thrombin generation, and magnitude of platelet adhesion. This approach reflects the multiple potential stimuli and pathways contributing to material thrombogenicity, including the intrinsic and extrinsic coagulation cascades along with platelet adhesion and activation.

When developing an *in vitro* dynamic test system to assess thrombogenicity, many factors such as the blood collection method, blood storage and handling conditions, test blood temperature, anticoagulation agents and concentration, flow conditions, and test duration need to be considered as they may affect the repeatability and reliability of the test results. Currently, there are several groups developing blood flow loop systems for the dynamic thrombogenicity evaluation of blood-contacting medical devices.[16–18] The aim of these systems is to replace or reduce the use of acute animal studies such as the NAVI model. A few developmental studies have shown that *in vitro* blood flow loop systems may be used to effectively compare the thrombogenic potential of biomaterials and devices with simple geometries.[16,18] Despite these promising results, more studies are needed to validate and optimize these systems before the methodology can be standardized.

Computational modeling of blood damage has the potential to improve the design and preclinical evaluation of medical devices. However, blood damage computational models need to be validated with preclinical or clinical data before they can be relied upon for regulatory decisions. FDA recently published guidance on the use and reporting of computational modeling in regulatory submissions.[29] As an ongoing research effort, the FDA *In Vitro* Blood Damage Assessment Laboratory has also partnered with industry, through the Medical Device Innovation Consortium (MDIC) program (www.MDIC.org), to develop experimental models to validate computational predictive models of hemolysis and thrombosis.[30]

Important Areas of Research

In this forum, the following research topics were identified as important areas that need to be investigated to improve preclinical device thrombogenicity evaluations:

(1) The clinical relevance of *in vitro* tests and computational models is currently not well-established. Studies need to be performed to correlate *in vitro* and *in silico* results with *in vivo* and clinical thrombosis data, and clinically relevant acceptance criteria for *in vitro* testing need to be determined.
(2) The applicability and limitations of acute *in vitro* testing to predict long-term outcomes of implantable medical devices also need to be determined.
(3) Best practices or standardized *in vitro* methods tailored to specific device types (*e.g.*, catheters, oxygenators, stents, VADs) need to be developed and validated. To achieve this aim, the key test parameters such as anticoagulation conditions, biomarkers, control materials and devices, sample size, and flow rates need to be determined. Various thrombogenicity markers (*e.g.*, visible thrombus, TAT, platelet count and platelet activation markers) need to be compared and selected to optimize the test sensitivity.

Table 1. Summary and Analysis of the Main Categories of Preclinical Thrombogenicity Testing

Type of Study	Strengths	Weaknesses	Important Areas of Research
Static *in vitro* tests (*e.g.,* PTT, platelet and leukocyte count, TAT)	Relatively simple test systems that tend to yield more consistent results	Unable to assess device thrombogenicity related to blood flow and surface geometry	Correlate the test results with *in vivo* and clinical thrombosis data
	Human blood can be utilized to account for species differences	Has very limited value in predicting long-term outcomes of implantable medical devices	Establish clinically relevant acceptance criteria
	May be sufficient to characterize device thrombogenicity related to the chemical properties of blood-contacting device materials	Clinical relevance of these tests is currently not well-established	Develop test methodologies with greater test sensitivity
	Could be used as screening tools to assist in the material selection for blood-contacting devices	May not be able to distinguish subtler thrombogenicity differences among commonly used biomaterials	Develop and standardize additional testing methods
		Only two complete test methods have been standardized and subsequently recognized by the FDA (ASTM F2888-19, ASTM F2382-18)	Evaluate the potential impact of the medical conditions and related medications on the way the blood responds
		Blood from healthy human subjects may not yield the same results as blood from the target patient population	
Dynamic *in vitro* tests (*e.g.,* blood flow loop assay)	Simulates typical clinical blood flow conditions	These acute tests may not be reliable in predicting long-term outcomes of implantable medical devices	Correlate the test results with *in vivo* and clinical thrombosis data
	May be appropriate to characterize device thrombogenicity related to blood flow and surface geometry of short-term blood-contacting medical devices	Clinical relevance of these tests is currently not well-established	Develop methods to quantify thrombosis and embolization
	Better control of test parameters (*e.g.*, flow rates) than in animal studies and often less burdensome	There are no standardized or widely accepted test methods (*e.g.*, test systems, anticoagulation protocols)	Determine applicability and limitations of acute *in vitro* testing to predict long-term outcomes of implantable medical devices
	Has the potential to replace or reduce some acute animal studies (*e.g.*, NAVI)	The use of human blood may not be feasible for some tests that require a large blood volume	Develop and validate best practices or standardized test methods tailored to specific device types (*e.g.*, catheters, oxygenators, stents, VADs)
			Ascertain what magnitude of change to device geometry and surface roughness increases the risk of thrombosis
In vivo tests (*e.g.*, Clinically relevant animal study, NAVI)	Simulate many aspects of clinical applications	Animal studies do not always accurately predict device thrombogenicity in humans	Identify the most clinically relevant animal species for specific medical device types
	Provide valuable information on device safety and performance	Species differences may make correlating animal results to human outcomes challenging	Establish clinically relevant anticoagulation protocols (based on ACT levels or develop a more robust method for determining blood coagulability) in different animal species
	Permit detailed measures of device function, blood-device interaction, and frequent in-life veterinary examinations of animals	Often more burdensome than *in vitro* or in silico testing	Optimize study protocols including the thrombosis scoring system
	Allow the opportunity to conduct detailed pathological evaluations after chronic use of a device	Animal studies are conducted on healthy animals, which may impact the translation of the results	Defining acceptance criteria Evaluate the usability of heart failure animal models
In silico simulation (*e.g.*, computational modeling of device-related thrombosis)	May overcome some limitations of *in vitro* and *in vivo* models and provide a better quantitative thrombosis assessment	Current models of thrombosis have not been fully verified and validated	Generate credible experimental data in different device models to validate numerical/computational simulations
	Has the potential to reduce the time and cost of designing medical devices	Clinical relevance of computational modeling is currently not well-established	Correlate the modeling results with *in vivo* and clinical thrombosis data
	Has the potential to reduce the need for animal studies	There are no standardized or widely accepted models for predicting device thrombogenicity	Verify and validate the predictive power of the numerical and computational models
	Often less burdensome than *in vitro* and *in vivo* testing		

ACT, activated clotting time; FDA, Food and Drug Administration; NAVI, nonanticoagulated venous implant; PTT, partial thromboplastin time; TAT, thrombin–antithrombin; VADs, ventricular assist devices.

(4) Research on the impact of geometry and surface roughness on device thrombogenicity are needed to ascertain what magnitude of change to these parameters increases the risk of thrombosis.
(5) *In vivo* animal models also need to be improved by identifying the most clinically relevant animal species for specific medical device types, establishing clinically relevant activated clotting time (ACT) levels or developing a more robust method for determining blood coagulability in different animal species, optimizing the thrombosis scoring system, and defining acceptance criteria.

Mechanisms for Collaboration With FDA

To facilitate getting safer medical devices to patients as quickly as possible, the FDA supports collective efforts to improve the utility and predictability of preclinical thrombogenicity evaluations. There are several mechanisms to interact/collaborate with the FDA on test method development. The FDA Medical Device Development Tool (MDDT) program encourages research and test laboratories, medical device developers and manufacturers, academia, and research organizations to submit proposals for tool qualification per the FDA MDDT Guidance Document.[31] A Research Collaboration Agreement (RCA) can be established with the FDA to collaborate on regulatory science issues with external parties. Moreover, organizations may also collaborate with the FDA through the Medical Device Innovation Consortium (MDIC) or Standards Development Organizations.[32] Stakeholders can also gain feedback from the FDA on their overall thrombogenicity strategy and new thrombogenicity test methods for assessing medical devices through the presubmission process (Q-Submission Program).[33]

Discussion

As a follow-up to the FDA Public Workshop held at the FDA in 2014,[34] this forum was held to further discuss the current status and challenges of preclinical thrombogenicity testing of medical devices with perspectives from FDA, industry, academia, and clinical personnel. Device thrombosis is a complex issue that has multiple contributing factors such as the coagulability of patients' blood, nonphysiological blood flow patterns generated by or associated with the device (*e.g.* supraphysiological shear stresses, blood recirculation, stagnation zones), and the exposure of blood to foreign materials.[1–3] It is not surprising that developing reliable, predictive thrombogenicity assessment methods is difficult. A summary and analysis of the main categories of preclinical thrombogenicity testing is presented in **Table 1**. In preclinical testing, it is not always possible to control all the different factors that could affect thrombus formation. Therefore, a challenge for the medical device community is to determine the most relevant experimental parameters to better control and improve thrombogenicity evaluations.

In general, animal models can provide useful information regarding the thrombogenicity of blood-contacting devices. However, these models often produce highly variable and sometimes misleading results. To improve animal studies, experimental parameters such as the type of animal model, implant location, blood coagulation, and blood-contacting duration may need to be tailored and optimized to the specific device type. For *in vitro* testing, the ideal test medium would be human donor blood from actual patients with cardiovascular disease. However, for many assays, it is not feasible or ethical to use this blood source. Therefore, identifying appropriate and reliable substitutes for patient blood (*e.g.*, healthy human blood, animal blood) is essential for developing more accurate assessments of device thrombogenicity.

Other aspects of testing need to be considered, such as device placement and surgical technique, as they may also influence the thrombotic potential of a device.[35–37] Kilic *et al.* reported the results from the PREVENTion of HeartMate II Pump Thrombosis through Clinical Management (PREVENT) study. This study was a multicenter prospective investigation to evaluate the effect of utilizing a uniform set of surgical and medical practices for VAD implantation on device thrombosis. Their analysis found that HeartMate II positioning at implantation could significantly impact the incidence of adverse events reported at 6 months.[35] Chiu *et al.* performed numerical simulations of the HeartMate II and the HeartAssist 5 (ReliantHeart Inc., Houston, TX) VADs implanted in various configurations and showed that the thrombogenicity potential of a device may be improved by optimizing the device design and the surgical implant configuration.[36] Thus, including an evaluation of device positioning and proposed medical management in the preclinical testing of a device may provide a more reliable prediction of device thrombogenicity and offer insight into successful anticoagulation strategies.

In conclusion, this forum highlighted the need for the development of improved device thrombogenicity assessments to better predict short- and long-term clinical outcomes. Due to the complex interaction between device flow, material properties, and blood reactivity, static tests to assess materials alone are generally not adequate to evaluate the thrombogenicity profile of a device. A substantial amount of research remains to be performed in the field of device thrombosis testing to identify the important experimental parameters and potential markers of thrombogenicity. Although *in vitro* assays and computational models have the potential to supplement or replace animal testing, critical studies still need to be performed to correlate *in vitro* and *in silico* results with *in vivo* and clinical thrombosis data. The medical device community needs to continue to work together and unite around standardizing approaches to thrombogenicity testing to increase patient safety by addressing the complex problem of device thrombosis.

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