Introduction

Nearly all of the components of the blood coagulation cascade and the fibrinolytic system have been either knocked-out or over-expressed in mice (1, 2). In addition, numerous genes encoding platelet, vascular endothelial cell, and vascular smooth muscle cell proteins have been targeted in mice. These genetically modified animals have provided tremendous opportunities to examine the functions of specific gene products in vivo and their roles in disease processes. The small size of mice also offers an advantage to investigators studying the in vivo effects of compounds that are expensive or difficult to produce in large quantities. Consequently, mice are being used with increasing frequency as a model of human hemorrhagic and thrombotic disorders. A variety of murine thrombosis models have been established by several investigators. This article will review these models and examine their utility and limitations in studying the complex process of thrombosis.

Arterial thrombosis models

Ferric chloride (FeCl3) injury

The ferric chloride method has been used to study the impact of a wide variety of genetic and pharmacologic interventions on microvascular thrombosis in mice. The advantages and limitations of different models are examined. Related topics of mouse anesthesia, phlebotomy, and in vitro hemostasis testing are also reviewed.

Keywords

Mouse or murine, animal models, thrombosis, haemostasis

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Theme Issue Article
to induce thrombus formation within the microcirculation, as
described below (6).

**Photochemical injury**
This method involves intravenous administration of a photo-
reactive substance, Rose Bengal, followed by illumination of an
exposed arterial segment with green light (540 nm) delivered
from a xenon lamp equipped with a heat-absorbing filter (7, 8).
Rose Bengal accumulates in the lipid bilayer of endothelial and
other cells (9). Exposure of Rose Bengal to green light triggers
a photochemical reaction that produces singlet oxygen and
promotes the formation of other reactive oxygen species that
damage the vascular endothelium and initiate thrombus forma-

tion. The photochemical injury model appears to induce more
subtle vascular injury than the ferric chloride model, and there-
fore the time necessary to form an occlusive thrombus is
generally longer with the former model than the latter. In our
experience, approximately 40 minutes is required to form a
completely occlusive thrombus in the carotid artery of C57BL/
6J mice that receive 10 mg/kg Rose Bengal. The photochemical
injury model has been employed by several investigators to
identify important roles for a variety of hemostatic and fibrino-
lytic factors in thrombus formation after vascular injury. Most investigators have studied thrombosis within a normal segment of the carotid or femoral artery. However, Eitzman et al. demonstrated that the photochemical injury method can be used to target vascular injury and thrombosis to an atherosclerotic segment of the distal carotid artery, thereby producing a model more reflective of atherosclerotic plaque rupture and thrombosis (10).

Attention to several factors is necessary in order to obtain reproducible results with the photochemical injury model. Rose Bengal should be administered to each animal by the same route (typically via the tail or jugular vein) and over the same period of time – usually as a bolus. The laser light source should be maintained at a constant distance, usually 5-6 cm, from the blood vessel. The time interval between Rose Bengal infusion and initiation of blood vessel illumination should remain constant. We accomplish this by illuminating the artery before the Rose Bengal is infused.

**Venous thrombosis models**

**Photochemical injury**
This method is performed as described for the arterial injury method, except that the jugular vein is illuminated (11). The time required to form an occlusive thrombus is determined by monitoring blood flow with a miniature probe.

Figure 3: Inferior vena cava (IVC) thrombosis model. (A) Visualization of IVC through dissecting microscope (arrow points to animal’s head). (B) Suture ligation of IVC and side branch to induce venous stasis. (C) Cross-section of IVC excised 6 days after ligation, demonstrating occlusive thrombus and infiltration of inflammatory cells into wall of vein and the thrombus (hematoxylin/eosin staining; mag. x 20).
Venous stasis
This model involves ligation of the inferior vena cava (IVC) to induce venous stasis and thrombosis (12). Mice weighing 20 to 30 grams are anesthetized by inhalation of isoflurane gas and a midline laparotomy is made. The small bowel is exteriorized and placed to the left of the animal. The IVC is exposed by careful blunt dissection while sterile saline is applied at regular intervals to the exteriorized bowel to prevent its desiccation. Non-reactive prolene suture (7-0) is looped around the IVC immediately caudal to the origin of the renal veins and ligated, and then cannulated at the level of the left renal vein with a 27 gauge needle.

Table 1: Selected anesthetic agents for murine surgical procedures.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Route of Administration</th>
<th>Dose</th>
<th>Duration of Anesthesia</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbital</td>
<td>IP or IV injection</td>
<td>40-60 mg/kg</td>
<td>Up to several hrs</td>
<td>Easily administered. Provides prolonged anesthesia.</td>
<td>Causes respiratory depression and hypothermia. Poor analgesia (pain relief).</td>
</tr>
<tr>
<td>Ketamine &amp; xylazine</td>
<td>IP injection. Combine agents prior to injection.</td>
<td>Ketamine: 80-120 mg/kg. Xylazine: 5-10 mg/kg. Can give 2nd dose of ketamine only (1/5 original) to prolong anesthesia.</td>
<td>30-90 min</td>
<td>Easily administered. Provides more analgesia than pentobarbital.</td>
<td>Shorter duration of anesthesia than pentobarbital.</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Gas inhaled in chamber or via nosecone</td>
<td>Brief anesthesia: 300 µl in 500 ml container. Prolonged anesthesia: Induction: 3-4%. Maintenance: 1-2%.</td>
<td>As long as animal exposed, up to several hrs.</td>
<td>Rapid onset and offset of action.</td>
<td>Calibrated vaporizer with O₂ source necessary for prolonged anesthesia. Operator needs to avoid exposure.</td>
</tr>
</tbody>
</table>

IP, intraperitoneal; IV, intravenous; SC, subcutaneous.

Figure 4: Blood collection from inferior vena cava (IVC). The surgically exposed IVC (visualized through a dissecting microscope; arrow points to animal’s head) is cannulated at the level of the left renal vein with a 27 gauge needle.
along with major side branches, to completely obstruct blood flow (Fig. 3). The laparotomy incision is closed in 2 layers with 4-0 or 5-0 non-reactive suture material and the mouse is allowed to recover from anesthesia. Two-six days later the IVC is harvested. The weight of the IVC segment in mg is divided by the length of the IVC segment in cm to determine the amount of thrombus formed. The excised venous segment can be embedded in paraffin to enable histologic analysis. Another model of IVC thrombosis involves tightening 2 sutures separated by 0.7 cm for 20 minutes (13). The thrombus is removed, rinsed, dried, overnight, and weighed.

**Mechanical trauma**

Pierangeli et al. described a model in which forceps were used to deliver a standardized “pinch” (1500 g/mm²) to the surgically exposed femoral vein (14, 15). Rather than measuring blood flow and occlusion time, these investigators used a miniature fiberoptic device to transilluminate the injured segment of vein while it was being visualized through a stereo microscope equipped with a closed circuit video system. Computer-assisted gray scale analysis was used to determine thrombus area and the kinetics of thrombus growth and dissolution over a 50 minute period.

**Microvascular thrombosis models**

**Epinephrine-collagen infusion**

This method involves intravenous administration of a solution of epinephrine (60 µg/kg) and collagen (0.06-0.5 mg/kg), usually via the jugular vein (13, 16). These substances induce systemic platelet activation and obstruction of the microcirculation. The endpoint measured is death.

**Intravital microscopy**

Several approaches have been reported for inducing thrombi in microvessels as they are visualized through a microscope,

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**Table 2: Summary of murine thrombosis/thrombolysis models.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Vascular Site</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial Thrombosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>Carotid or femoral artery</td>
<td>Simple, inexpensive</td>
<td>Severe, “outside-in” injury</td>
<td>3-5, 28</td>
</tr>
<tr>
<td>Photochemical</td>
<td>Carotid or femoral artery</td>
<td>“Inside-out” injury</td>
<td>RB can be toxic to recovering mice.</td>
<td>9-11</td>
</tr>
<tr>
<td><strong>Venous Thrombosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photochemical</td>
<td>Jugular or femoral vein</td>
<td>Thrombus forms relatively quickly in a patent artery.</td>
<td>RB can be toxic to mice recovering from anesthesia.</td>
<td>11</td>
</tr>
<tr>
<td>Stasis</td>
<td>IVC</td>
<td>Model relevant to human DVT.</td>
<td>Ligature completely obstructs blood flow.</td>
<td>12, 13</td>
</tr>
<tr>
<td>Mechanical trauma</td>
<td>Femoral vein</td>
<td>Measures kinetics of clot growth</td>
<td>Utilizes specialized equipment.</td>
<td>14, 15</td>
</tr>
<tr>
<td><strong>Microvascular Thrombosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic epinephrine-collagen infusion</td>
<td>Systemic microthrombosis</td>
<td>Easy to perform. Useful for studying anti-plt agents.</td>
<td>Component of vascular injury is absent.</td>
<td>13, 16</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>Mesenteric arterioles</td>
<td>Studies plt-plt and plt-wall interactions.</td>
<td>Requires ex vivo platelet labeling.</td>
<td>6</td>
</tr>
<tr>
<td>Laser injury</td>
<td>Small car veins and arteries</td>
<td>Non-invasive. Can induce multiple thrombi/animal, apply to larger veins, and combine with RB injury.</td>
<td>Requires specialized equipment. Difficult to reproducibly induce arterial thrombi by direct laser injury.</td>
<td>18</td>
</tr>
<tr>
<td>Laser injury with fluorescent Ab imaging</td>
<td>Cremasteric muscle arterioles</td>
<td>Real-time imaging of fibrin, plt, TF.</td>
<td>Requires specialized equipment/reagents.</td>
<td>19</td>
</tr>
<tr>
<td><strong>Thrombolysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous lysis of pulmonary embolism</td>
<td>Preformed clot injected in jugular vein</td>
<td>Good model of a major human disease.</td>
<td>Technically difficult. Results can be variable.</td>
<td>20</td>
</tr>
<tr>
<td>Spontaneous lysis of pulmonary microemboli</td>
<td>Fibrin microparticles injected into tail vein</td>
<td>Tail vein injection less invasive than jugular vein injection.</td>
<td>Requires in vitro processing of clot to prepare microparticles.</td>
<td>21</td>
</tr>
<tr>
<td>Pharmacological arterial thrombolysis</td>
<td>Carotid artery</td>
<td>Involves lysis of plt-rich thrombus formed in situ.</td>
<td>Murine Plg is resistant to bacterial &amp; human PAs.</td>
<td>5</td>
</tr>
</tbody>
</table>

**Notes:**

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thereby allowing real-time imaging of thrombus growth and dissolution. Denis et al. described a method in which ferric chloride is used to induce thrombosis within mesenteric blood vessels of the small intestine (6). For this procedure, platelets are isolated from a donor mouse and fluorescently labeled ex vivo. Another mouse is anesthetized and the labeled platelets are injected into the tail vein. A midline abdominal incision is made and the mouse is laid on its side on the platform of a fluorescence microscope that is connected to a video camera with recorder. The small intestine is exteriorized and an arteriole (diameter 60-100 µm) within the mesentery is visualized. Thirty µl of ferric chloride solution (250 mM) is placed on the surface of the mesentery overlying the visualized arteriole. Platelet deposition on the arteriolar wall, occlusive thrombus formation, and embolization of thrombi can be visualized, recorded, and quantified. Andre et al. extended this technique by utilizing computer software to continuously measure fluorescence intensity of injured mesenteric arterioles, thereby providing a more quantitative assessment of thrombus growth in real time (17).

Rosen et al. used 2 forms of laser injury to study thrombosis within small veins of the mouse ear (18). Direct laser injury of veins with diameters ranging from 100-350 µm can be induced by applying short bursts of a high-energy argon-ion laser to the luminal surface of the blood vessel. Alternatively, Rose Bengal can be injected intravenously and photochemical injury and thrombosis can be triggered by focusing the laser on a vein. Video recordings of the evolving thrombus are captured, digitized, and analyzed with a computer software package to measure thrombus area and integrated optical density every few seconds. Interestingly, analyses of the effects of coagulation and platelet inhibitors on thrombus growth suggested that the 2 forms of laser injury induced thrombosis by different mechanisms. Whereas thrombi induced by direct laser injury appeared to involve mainly platelet interactions, thrombosis triggered by photochemical injury appeared to depend on both platelet interactions and activation of the coagulation cascade.

Falati et al. used confocal and widefield microscopy to study thrombus formation within cremasteric muscle arterioles (diameter 30-60 µm) (19). A pulsed nitrogen dye laser (440 nm) is used to induce vascular injury. Thrombus growth and dissolution is imaged, recorded, and analyzed with a digital camera and computer workstation. As a component of this model, these investigators infuse into mice fluorescent antibodies that are specific for tissue factor, fibrin, and platelets to enable real-time measurement not only of thrombus size, but also specific thrombus components.

**Thrombolysis models**

Several approaches have been used to study clot lysis in the living mouse. Carmeliet described a method in which plasma clots containing 125I-fibrin are formed in vitro and injected into the jugular vein of an anesthetized mouse (20). The clot embolizes and lodges within the pulmonary vasculature, where it gradually lyases. Several hours later the animal is euthanized. The heart and lungs are removed en bloc and their radioactivity (counts per minute) is measured. Since the radioactivity of the clot is measured before injection, % clot lysis can be measured. Bdeir et al. described a related method in which radiolabeled fibrin microparticles are formed in vitro and injected into the tail vein (21). Pharmacologically-induced arterial thrombolysis can also be studied in mice (5). An occlusive thrombus is induced in the carotid artery by ferric chloride administration. The time required to achieve reperfusion after administration of a plasminogen activator (PA) is measured. Since murine plasminogen is relatively resistant to activation by human and bacterial PAs (e.g. human tissue-type plasminogen activator and streptokinase, respectively) purified human plasminogen is administered along with the PA in order to temporarily “humanize” the murine plasminogen activation system. Transgenic mice expressing human plasminogen could be used to obviate the need for administering purified human plasminogen when studying pharmacologic thrombolysis.

**Anesthesia**

Mice must be anesthetized for periods ranging from minutes to hours in order to perform thrombosis protocols. Tracheal intubation and mechanical ventilation is not required for the models described in this review. While invasive (e.g. femoral artery catheter) or non-invasive (e.g. tail blood pressure cuff) monitoring of systemic blood pressure during surgery might be desirable for some experiments, it is generally not performed. Anesthetic agents are administered to mice either parentally or by inhalation (Table 1). Parenteral anesthetic agents can be delivered by subcutaneous, intraperitoneal, or intravenous injection. Intramuscular injections should not be given to mice. Inhalation agents are administered either within a chamber or by a facemask. The former approach involves placing anesthetic-soaked cotton or gauze within an airtight container. The mouse is placed within the container (but not in direct contact with the anesthetic) and removed after it becomes unresponsive. This approach is suitable only for short-term procedures, such as phlebotomy, since the mouse recovers quickly as it breathes room air. For a longer surgical procedure, inhalation anesthesia with agents such as isoflurane or halothane must be administered with supplemental oxygen via a precision vaporizer and facemask. Inhalation anesthesia with isoflurane is the preferred method of anesthesia for mouse surgical procedures. Adjunctive agents can be administered along with the anesthetic. For example, atropine (0.05 mg/kg) can be administered by subcutaneous injection as a preanesthetic agent to prevent the decrease in heart rate and excessive salivation that some anesthetics can induce. Because it has a relatively large body surface area/mass ratio, the mouse is prone to hypothermia while under general anesthesia. To minimize body heat loss, the mouse
should be placed on a heating pad during and after any surgical procedure. If necessary, a thermal lamp or subcutaneous or intraperitoneal injections of warmed saline can be used to prevent post-operative hypothermia. To prevent injury, an anesthetized mouse should not be caged with other mice until after it is fully ambulatory.

Phlebotomy, platelet function assays, and in vitro hemostasis testing
As a component of thrombosis studies, it is often necessary to collect blood for in vitro studies. Given their size, it can be challenging to collect blood from mice and to process it in vitro in a manner that yields the high quality samples typically necessary for hemostasis assays. Blood can be collected relatively easily from the retroorbital venous plexus into a glass capillary tube. However, this method yields only approximately 200 μl of blood, and the probing that is necessary to cannulate the venous plexus usually leads to mild tissue maceration that can expose blood to tissue factor, leading to some degree of activation of the coagulation cascade. Blood can be collected from the surgically exposed inferior vena cava via a needle (Fig. 4). By this approach, 0.5-1.0 ml of blood can usually be obtained from an adult mouse. We induce CO₂ narcosis immediately before surgically exposing the IVC, since this form of euthanasia facilitates phlebotomy by producing venous dilation. Blood can also be obtained directly from the surgically exposed heart. Sodium citrate is most commonly used to anticoagulate blood in vitro. Protocols for performing prothrombin time and activated partial thromboplastin time assays are included in the appendix.

Assessment of platelet function is often an important component of thrombosis studies. Determining the bleeding time is a simple, yet effective method for assessing platelet function in vivo (22, 23). Ex vivo perfusion chambers are useful for assessing platelet activation and deposition under conditions of defined flow rate and shear stress (17, 24). Standard in vitro platelet aggregometry remains an effective method for assessing platelet responsiveness to a variety of agonists. Protocols for preparing platelet-rich plasma and performing in vitro platelet aggregometry are included in the appendix.

Applications, advantages, and limitations of murine thrombosis models
From the preceding discussion it is evident that multiple options are available to the investigator interested in modeling thrombosis in the mouse (Table 2). Several factors must be taken into account when deciding which model to employ, including the expense and availability of specialized equipment, the technical expertise required, and the disease process that is of interest. For example, an investigator focusing specifically on platelet-platelet interactions might consider using the epinephrine-collagen infusion model, whereas investigators interested in the pathogenesis of macrovascular arterial vs. venous thrombosis might consider the carotid injury and IVC ligation models, respectively. Intravital microscopy approaches are particularly well suited to those interested in studying thrombus formation within the microcirculation. It is important to remember that the impact of a particular gene or compound on thrombosis may be markedly affected by a variety of factors, including the vascular site being studied, the rate and character of blood flow (e.g. turbulent vs. laminar), and the type and severity of vascular injury that is used to trigger blood clotting. Experience is also a key determinant of experimental results. To successfully and reproducibly perform microsurgical procedures on anesthetized mice the operator must be prepared to complete a learning curve that may consist of as many as 30-50 experiments.

All thrombosis models have potential limitations. Many of the methods that have been used involve triggering thrombus formation within a normal blood vessel. However, pathologic thrombosis in humans, e.g. as in myocardial infarction, usually occurs within a diseased vascular segment. Some of the endpoints of thrombosis studies, e.g. survival rates and the time required to form a completely occlusive thrombus, are relatively crude, and therefore may not detect subtle yet real effects of the genetic modification or therapeutic compound under study. Many of the stimuli used to trigger thrombosis in mice, such as ferric chloride application to an artery or complete ligation of a vein, do not ideally model the triggering mechanisms responsible for thrombosis in human diseases. In addition, it is possible that the methods used to trigger thrombosis, such as chemical or thermal injury, may denature proteins within the blood vessel wall, thereby limiting the ability to study their role in thrombosis.

Despite these potential limitations, mice have become a key species for the laboratory-based investigation of the pathogenesis, prevention, and treatment of thrombosis. The driving force behind this transition from traditional larger animal models has been the explosive growth of molecular biology approaches to altering the mouse genome, thereby allowing analysis of gene function in vivo. Histologic analyses of murine thrombi reveal that they are structurally similar to, and often indistinguishable from, thrombi that form in humans (4). Genetic over-expression and deletion of various activators and inhibitors of blood clotting have produced phenotypic changes in mice that generally are concordant with those observed in humans with spontaneous mutations of the corresponding factors. In addition, anti-coagulant and antiplatelet drugs that are used clinically generally inhibit thrombosis in mice, indicating that the mechanisms of thrombus initiation and growth in murine models are similar to those in humans. These observations support the use of the laboratory mouse to study human thrombotic disorders. However, significant species differences exist, and these may
influence the interpretation of experimental data and their relevance to human physiology and disease, as has been nicely reviewed elsewhere (1). Murine platelet counts are approximately 4-fold higher than those of humans, while the mean platelet volume of mice is approximately half that of humans. The reactivity of murine platelets to some agonists, such as epinephrine and serotonin, is significantly reduced compared to human platelets, and there are differences in the thrombin responsiveness of members of the protease activated receptor family between mice and humans (25). It is also important to realize that significant differences in thrombotic tendency may exist between different mouse strains.

**Future directions**

It will be important to continue to improve the methodology that is used to study thrombosis in mice. From a technical perspective, it will be very useful to further develop practical, relatively inexpensive methods for quantitatively measuring the rate of clot growth in larger arteries and veins (14, 15). The ability to determine the kinetics of clot growth and lysis, as opposed to the simpler endpoints of occlusion and reperfusion times, will enable more precise and sensitive experiments. It also will be important to develop more clinically relevant thrombosis models, a process that has been accelerated by molecular genetic approaches. For example, the factor V Leiden mutation has been introduced into mice (26). These animals exhibit a pro-thrombotic state that should prove useful for studying other genes that modify the phenotype associated with this disorder (27), as well as new antithrombotic drugs. In addition, the capacity to cross hyperlipidemic mice, such as those lacking apolipoprotein E or the low density lipoprotein receptor, to mice with targeted mutations in other genes has proved a powerful tool for studying the genetic determinants of thrombus formation within an atherosclerotic artery (10). Lastly, it will be important to develop better murine models to screen genetically modified mice for pro-thrombotic phenotypes. For example, random mutagenesis can be used to identify, in a non-biased fashion, genes that modulate thrombosis. Such approaches will rely on assays that can rapidly and inexpensively screen a large number of offspring for potential pro-thrombotic phenotypes.

**Appendix**

**Platelet aggregometry**

1. Count platelets using a Particle Counter (Beckman Coulter, Miami, FL) or similar device.
2. Dilute PRP with PPP to adjust platelet count to 2.0-2.5 × 10^11/ml.
3. Add 225 μl of count-adjusted PRP to a 7.25 × 55mm flat-bottom glass tube containing a magnetic stir bar. Incubate platelet suspension without stirring at 37°C for 5 minutes prior to studying aggregation.
4. To obtain platelet-poor plasma (PPP), centrifuge remaining blood at 1500 × g for 15 minutes at room temperature. Collect PPP in separate tube. Can be frozen at -80°C

**Prothrombin time (PT)**

1. Clotting assays can be performed manually or with an automated instrument. We use the Amelung KC4 Delta Micro-Coagulation Analyzer (Trinity Biotech), which detects clot formation by sensing loss of motion of a magnetically stirred metal bead placed in the clotting mixture. Several other automated devices are commercially available.
2. To get a 3-fold activated partial thromboplastin time (APTT) reagent (Organon Teknika Corporation, Durham, NC), prior to use, we dilute the APTT reagent 3-fold in Tris-buffered saline to produce a longer clotting time.
3. Add 80 μl of prewarmed PT/Cl mixture, which is prepared by mixing equal volumes of PT reagent (Organon Teknika Co.) and 30mM CaCl2.
4. Record time to form a clot.

**Activated partial thromboplastin time (APTT)**

1. Add 40 μl of 3.8% sodium citrate anticoagulant into a 1 ml syringe. This assumes collection of at least 360 μl blood (400 μl total volume).
2. Collect maximum amount of blood possible from mouse IVC or left ventricle, using a 25- or 27-gauge needle.
3. Remove needle from syringe, carefully expel any excess air, and note total volume in syringe.
4. Calculate the volume of citrate needed to adjust the final blood-anticoagulant ratio to 9:1.
5. Empty blood into 1.5 ml polypropylene tube and add additional citrate. Mix well.

Note: If it is possible to routinely collect >360 μl of blood per mouse, then the volume of anticoagulant placed within the syringe can be increased.

**Preparation of platelet-rich and platelet-poor plasma**

1. Blood samples from individual mice can be processed separately or pooled prior to centrifugation.
2. Centrifuge citrated blood in 1.5ml polypropylene tubes, in a swing-out rotor, at 120 × g for 8-10 minutes at room temperature.
3. Carefully remove the platelet-rich plasma (PRP). To maximize platelet recovery, the remaining blood can be centrifuged again for 3-5 minutes and the additional PRP collected.
4. To obtain platelet-poor plasma (PPP), centrifuge remaining blood at 1500 × g for 15 minutes at room temperature. Collect PPP in separate tube. Can be frozen at -80°C.

**Phlebotomy**

1. Pipette 40 μl of 3.8% sodium citrate anticoagulant into a 1 ml syringe. This assumes collection of at least 360 μl blood (400 μl total volume).
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3. Remove needle from syringe, carefully expel any excess air, and note total volume in syringe.
4. Calculate the volume of citrate needed to adjust the final blood-anticoagulant ratio to 9:1.
5. Empty blood into 1.5 ml polypropylene tube and add additional citrate. Mix well.

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