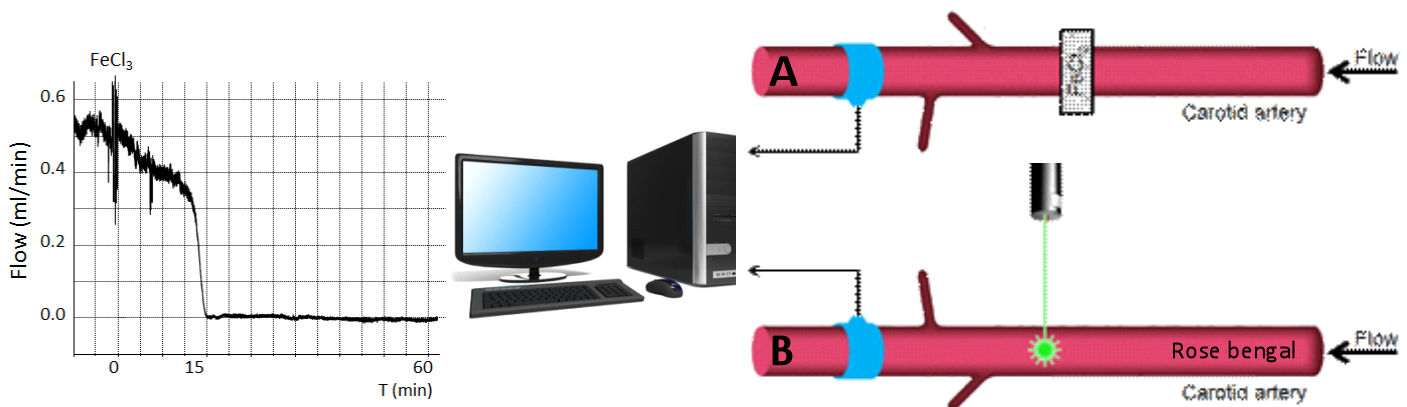


Thrombosis, the obstruction of blood flow due to clot formation, may result in tissue anoxia and damage, and it is a major cause of morbidity and mortality worldwide. In the nondiseased state, physiologic hemostasis reflects a delicate interplay between factors that promote and inhibit blood clotting, favoring the former. This response is crucial as it prevents uncontrolled hemorrhage following injury. In specific settings, the same processes that regulate normal hemostasis can cause pathologic thrombosis, leading to arterial or venous occlusion, and the severity of the clinical manifestation depends on the affected vascular bed.

Many commonly used therapeutic interventions may alter the thrombotic–hemostatic balance adversely. Animal models are used to understand the pathological pathways involved in thrombosis and to test the efficacy and safety of new drugs (thrombotic preventive/protective role) that could be also assessed for hemorrhagic complications using animal models of bleeding.

- ✓ **Ferric chloride induced carotid artery injury model:** Mice are anesthetised using a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine that is i. p. administered at the beginning of the experiment. A Doppler flow probe is placed around the left carotid artery and a 2×2 mm piece of Whatman paper soaked in 15 % ferric chloride is applied to the artery. The Whatman paper is removed after 5 min and the vessel washed with saline. Flow through the carotid artery is monitored with the Doppler flow probe and vessel occlusion is considered to occur at the time when flow is totally interrupted and flow cessation lasts for 30 min. When there is no thrombus formation within 40 min, this is considered to be the vessel occlusion time for calculation purposes.



Scheme showing ferric chloride (A) and Rose Bengal/laser (B) induced carotid thrombosis.

- ✓ **Rose Bengal/laser induced carotid artery injury model:** After applying anesthesia with the mixture of ketamine and xylazine, the left common carotid artery was fitted with a Doppler flow probe (Model 0.5 VB; Transonic System, Ithaca, NY, USA). Then, 100 mg/kg Rose Bengal was i. v. infused and the carotid artery immediately exposed to 1.5-mW 540-nm laser light. Flow through the carotid artery was monitored with the Doppler flow probe and vessel occlusion was considered to occur at the time at which flow was totally interrupted and after which flow cessation lasted for 30 min.
- ✓ **Lung thromboembolism model:** After applying anesthesia with the mixture of ketamine and xylazine, massive thromboembolism to cause respiratory arrest is then induced by an i. v. injection of a mixture of 0.8 mg/kg collagen and 60 µg/kg epinephrine. Animals that remain alive 30 min after challenge are considered survivors.
- ✓ **Tail bleeding model :** Mice are anaesthetized with 2.5% isoflurane, and maintained at 37°C on heating pads. 5 mm of tail tip is removed using a scalpel blade and the tail tip bathed in 1 ml of sterile saline at 37°C. The time to cessation of bleeding will be measured up to 30 min.