Performing flow-based assays under coagulation conditions

Biorheology Subcommittee

Person responsible (Chair / Principal Investigator): Mikhael Panteleev and Pierre Mangin

Description Abstract

**Background:**

*In vitro* flow based assays have been central in providing a better understanding of the processes of platelet adhesion, activation and aggregation both at the cellular and molecular levels. They can also be important tools to evaluate the effect of potential anti-platelet agents. However, the vast majority of experiments based on this technique are performed using anticoagulated whole blood, which only mimics the process of primary hemostasis and undervalues the role of coagulation in arterial thrombosis. As primary and secondary hemostasis are fully interdependent, it seems of crucial importance to investigate platelet function under conditions of coagulation, i.e. when thrombin is generated and fibrin is formed. Using experimental conditions which allow to study platelet adhesion, activation and aggregation in the presence of thrombin generation represents an important step forward to better delineate *in vitro* the mechanism of hemostasis or arterial thrombosis as they occur *in vivo*. The reason why investigators in the field have mainly used anticoagulated blood is most likely related to the challenge of performing such experiments which are of high technical complexity.

**Objective:**

The main objective of this work is to describe and discuss the experimental conditions which are used by experts in the field to study thrombus formation under flow in the presence of thrombin generation.

We will provide a manuscript in which:

We will start by discussing the advantages and relevance of using flow models in which coagulation and/or platelet function can be studied under hemodynamic conditions found in hemostasis or under venous and arterial thrombosis. We will provide an overview of the current techniques that can be used to initiate coagulation in a flow device, mention their advantages and the current limits.

Specific issues to be particularly addressed will include:

1) We will describe all devices that aim to properly mix blood and calcium prior to flow chamber when citrated whole blood is used. We will discuss their limits and propose the
best suited tools allowing ideal mixing as well as review possible alternatives to dynamic re-calcification.

2) Proper activation of coagulation, importance of the limited-size activators specifically in coagulation, and challenges associated with immobilizing and standardizing tissue factor instead of (or together with) collagen will be discussed.

3) A major issue for coagulation-based flow models is artificial contact activation of clotting by tubing and flow chamber surfaces. In non-flow assays (like thrombin generation assay), it can be prevented by corn trypsin inhibitor and similar molecules. However, for flow assays, this could inhibit the role of contact pathways of thrombosis.

4) In contrast to platelets (which are 1000-fold concentrated and thrombi and easily labeled), fibrin concentration in thrombus is not greatly different from that of fibrinogen in blood, the fibrin molecule is very similar to that of fibrinogen, and thrombin is rapidly diffusing and moved by flow. Therefore, issues of imaging coagulation in the flow models will be discussed.

5) Major limitations of the existing models (e.g. thrombomodulin on vessel walls, etc.) will be listed.

6) Typical changes in the chamber geometry (importance of avoiding stagnant regions), time and space scales, and overall experimental design will be considered.

7) Changes in analytical and pre-analytical variables compared with platelet-only flow assays using anticoagulated blood will be analyzed.

We will describe some original flow chamber well suited to perform flows under non anticoagulated conditions. We will discuss their advantages and disadvantages of these different approaches depending on the scientific question raised by an investigator. We will provide a series of recommendations to perform flows in the presence of thrombin generation to best suit the scientific question raised by an investigator.

Design and methodology (Data expected to collect, sample size and statistical analysis):

Describe concisely the research design and methods for achieving these goals. Suggested length 2-3 paragraphs

N/A

Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

N/A
SSC Subcommittee Project/Collaborative Project

Expected timeline:

- Project stage/set up:
  - Launch: **July 2020**
  - Duration: **1 year**
  - Finalization/analysis:
  - Reporting

Expected outcomes (ie. publications):

- Publication type (SSC Communication, Guidance document or original article):
  - recommandation article

Description of project set/up and management, needed infrastructure and resources (summary):

Possible references: