Influence of Surface and Fluid Conditions on Thrombus Generation

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Preface

A color film has been prepared for showing at normal sound speed for 16 mm movie projectors. The events portrayed are seen at three times the actual rate of occurrence. For example, each 20 seconds of movie time represents one minute of real time of blood flow over the sample surface. Experimental details of the surgical preparation used and the microcinematography apparatus employed are available. These details, and conclusions drawn from inspection of numerous film sequences such as those portrayed here, with abundant supporting data from simultaneously applied ancillary techniques, have been previously published with co-authors involved in the original experiments and cinematography.

Experimental Conditions

A stagnation point flow chamber, illustrated in Figure 1, was constructed in such a manner that only fresh axial-flowing blood from the jugular vein or carotid artery of a healthy, lightly-anesthetized animal would reach the surface under observation. The shear rate of the blood at the surface under observation was controlled at a value of 60 inverse seconds, approximating average venous shear rates, by adjustment of a pair of withdrawal syringe pumps drawing blood from the exit ports of the T-shaped stagnation point chamber. The flow chamber was always scrupulously cleaned and filled with physiologic saline prior to the application of transparent test materials to the upper face of the chamber. The saline replaced all air adjacent to the original material face prior to admitting blood. As blood was allowed to enter the chamber, the saline was rapidly, but not instantaneously, displaced.

Experiments with chambers constructed of differing materials, including materials bearing an adsorbed coating of the natural anticoagulant heparin, demonstrated that no influence of the material or chamber construction was detectable at the sample surface under observation. The microcinematography apparatus was generally focused on the blood contacting interface of the sample surface just downstream from the line of blood flow bifurcation (that is, the stagnation point). A mercury lamp was used to obliquely illuminate the test surface and the back-scattered light was collected by a microscope objective. (See Figure 2) At the conclusion of each experiment, the surface under observation was removed from the chamber and examined by a variety of light microscopic techniques as removed and after histologic staining and sectioning. Generally, 15 minutes of exposure of the sample surfaces to fresh flowing blood was sufficient to deposit one mm thick thrombus on the blood contact face of the sample. This thrombus mat most often consisted of clearly isolated 50 to 200 micrometer diameter aggregates of platelets and white cells (usually segmented neutrophils) adherent to a spontaneously adsorbed protein film (of about 200 Angstroms thickness) directly in contact with foreign material. The platelet aggregates had pyramidal shapes, with their apexes towards the original flowing blood volume. Between the aggregates, a fibrin mesh with numerous en-
trapped red cells was found in all cases in which the blood remained free of anticoagulants.

Film Sequence One

The film opens with the surgical dissection of the canine jugular vein and its preparation for mounting to the stagnation point flow chamber. A ring with a diameter approximately equal to that of the blood vessel is slipped over the dissected vessel end. Then, with the use of hemostats, the vessel itself is everted over the ring. This provides a mechanical support to the vessel and hides the cut edges of the blood vessel from the flowing blood. The ring-supported vessel is then placed into a cylindrical opening in the bottom of the stagnation point flow chamber and its freedom from occlusion noted by allowing a small amount of blood to flow. The entire chamber is rinsed and the test surface of interest is mounted at the upper face of the flow chamber. The everted blood vessel is located centrally with respect to the test surface, usually a 1" × 3" glass slide or similarly sized portion of transparent material.

After assembly, the chamber is completely filled with saline, and the upper sample surface is flushed and wiped free of dust. This operation is never completely successful in eliminating dust particles, which particles are then used as reference points since they appear in each photographic frame. An optical microscope is placed over the test chamber and arranged for oblique illumination with a bright mercury lamp.

The first film sequence demonstrates the events at one of the best test surfaces available, a polydimethylsiloxane-coated glass slide. (See Figure 3) Flow, in the film, is from the bottom to the top of the screen. Attention is drawn to the small thrombogenic nidus in the center of the screen. This had been identified, during the experiment, as a small bit of residual debris on the blood contact side of the plate which held an air bubble in place. The time of observation shown is from one minute through 14 minutes after the beginning of the experiment. It will be noted that a diffuse motting of adherent platelets exists over the entire sample surface at the one minute observation time. The large white objects which remain static on the outer surface are the dust particles which had not been previously removed. As blood flow continues, platelets accumulate into an aggregated mass at the central nidus and further begin to perturb the blood flow in their immediate vicinity. A typical white thrombus, dominated by platelets but with a large number of white cells joining in, as well, grows in a wake pattern upstream.

The field of view is 1.5 mm × 1 mm at this magnification. Blood flow continues with no aggregation on one side of the screen but there has been a significant reduction in the flow at the other side which results from a thrombus mass which was generated slightly upstream of the area under observation. Once the thrombus has begun to three-dimensionalize into the blood flow, the fluid mechanical perturbations tend to catalyze even more significant growth and deposition of platelets, aggregates, and white bodies. After 14 minutes of blood flow over this well-methylated glass surface, typical of the best biomaterial surfaces ever exposed to blood, the surface flow in the central portion of the sample under observation is markedly retarded. In particular, a large collection of white cells has formed in a central circle around the thrombus mass. Along the margin of the microscopic field shown at the end of the film sequence, it can be seen that the flow adjacent to the surface had ceased completely. When the plate was removed from the chamber and fixed and sectioned for routine light microscopy, a red cell/fibrin mesh was found in this area. (See Figure 4)

Film Sequence Two

Again in this film sequence, the field under observation is 1.5 mm × 1 mm. The surface under observation is an acid-cleaned, distilled water-rinsed soda lime glass plate. The film sequence characterizes typical conditions on such clean glass surfaces at about ten minutes after first contact with fresh flowing blood. The actual film sequence covers the elapsed experimental time from eight minutes through 11 minutes after first blood contact. It is seen at the initiation of this sequence, that by eight minutes the entire clean glass surface is covered with platelet aggregates, and a nearly
complete interaggregate mesh of fibrin with its entrapped red cells has already developed. In regions between the thrombotic deposits of platelets and white cells, motion of blood flowing well beneath the blood/glass interface can be detected. (See Figure 4)

![Figure 4](image.png)

Transmission electron microscopy of sectioned thrombus showing platelet/neutrophil thrombus and red blood cells trapped in fibrin at top. Thrombus at this stage is pyramidal-shaped and disturbs blood flow in the immediate vicinity.

**Film Sequence Three**

Improvements in the optical components utilized allowed filming of sequences at even higher magnifications. In this film sequence, the field of view characterized is 150 micrometers by 400 micrometers. The sequence demonstrates the initial events of platelet deposition and secondary white cell adhesion from the instant before first contact with fresh flowing blood to a time 3.5 minutes later. This sequence is atypical in that more rapid cell deposition is shown than normally occurred. Inspection of numerous such sequences demonstrated that platelets, although arriving at the surface from the initiation of first blood contact, did not adhere to the foreign material (already coated with a spontaneously deposited proteinaceous film) until between 40 and 60 seconds of continuous blood flow had taken place. Similarly, white blood cells were not generally noted to adhere until after four to six minutes of continuous blood flow.

In this film sequence, however, where the foreign material was freshly acid-cleaned soda lime glass, it is seen that immediately after displacement of the physiologic saline (apparent by its light green color) platelets begin to deposit randomly within 20 seconds and thence, rapidly form aggregates with arriving neighbors. White blood cells are already beginning to adhere before the film sequence ends after some 3.5 minutes of actual flow time.

The initial processes of platelet adhesion, aggregation and full thrombus formation are quite well visualized. This film sequence characterizes events immediately adjacent to the stagnation point where the fresh flowing axial blood from the living animal was first contacting the slide.

**Film Sequence Four**

In this sequence, the field of view is again 250 micrometers by 400 micrometers and the foreign material under observation is acid-cleaned soda lime glass. The optical apparatus was repositioned, however, to record the events a few millimeters downstream from the line of flow bifurcation. The period of blood contact documented in this sequence is from the instant before initiation of blood flow to eight minutes after that initiation. Close inspection of this film sequence demonstrates that an apparently random deposit of a first layer of platelets occurs at about one minute, but, in contrast to points nearer the line of flow bifurcation, a significant delay in aggregation is noted. A prolonged period (of about four minutes) is recorded prior to the almost explosive growth of aggregates at this downstream region on the acid-cleaned glass surface, accompanied by significant numbers of adhering white blood cells (beginning at about 4.5 minutes of real elapsed time).

It is clear that it took a very long time for displacement of the original physiologic saline solution from the downstream surface, but careful inspection of static frames from this film sequence reveal that within the first minute of actual blood flow platelet deposition in a singular fashion had already occurred. Once a particular threshold concentration of some unknown substance has occurred at the interface, the deposition process proceeds in almost avalanche fashion. Platelet thrombi and adjacent white cells (primarily segmented neutrophils) shut off the near-surface blood flow in less than a minute. As can be seen at the margin of the field of view, subsurface blood flow continues undisturbed throughout the period under observation. A coherent platelet and white cell mass, the typical white thrombus, is shown at the termination of this film sequence.

**Film Sequence Five**

The final sequence is an appended example of numerous lower magnification films taken in a direct-flow-through chamber similar to that later used in the investigations of Friedman and co-workers. The foreign material under observation is a slab of detergent-washed, distilled water-rinsed polymethylmethacrylate, a nominally thrombogenic material, a few minutes after first contact with fresh flowing blood. This film sequence is included to demonstrate, over a larger surface area, the growth of individual clumps of platelets and white cells along with the intervening fibrin mesh and entrapped red cells.

**References**

