

How shear modulates fibrin assembly: A microfluidic SAXS perspective

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Summary. — We present a SAXS-compatible microfluidic platform for studying fibrin polymerization under controlled shear. The device enables *in situ* monitoring of early stages of assembly while allowing precise tuning of shear conditions. SAXS measurements comparing low (10s^{-1}) and moderate (500s^{-1}) shear reveal reproducible differences in protofibril organization, showing that flow can influence nanoscale assembly and establishing microfluidic SAXS as a robust tool for studying dynamic, shear-sensitive protein polymerization.

1. – Introduction

Fibrinogen (fig. 1(A)) is a key, highly dynamic, and flexible plasma glycoprotein that is essential for blood coagulation, tissue repair, and immune response [1]. This structural flexibility allows exposure of binding sites that regulate polymerization. Upon thrombin cleavage, fibrin monomers assemble into two-stranded protofibrils, which subsequently aggregate laterally into fibers and form a three-dimensional network (fig. 1(B) and (C)) [1]. This hierarchical assembly, spanning nanometers to micrometers, governs clot structure and mechanical properties.

The conversion of fibrinogen into fibrin represents the final step of the coagulation cascade and is essential for hemostasis. Disruptions in this process are associated with pathological conditions such as thrombosis and other non-vascular diseases [1, 2]. Although the biochemical pathways of fibrin formation are well studied, the physical mechanisms that control fibrin assembly are only partially understood [1]. Physiological flow introduces additional complexity: shear influences collision rates, protofibril orientation, and early aggregate stability [3]. However, the effects of moderate shear on nanoscale protofibril organization remain unclear.

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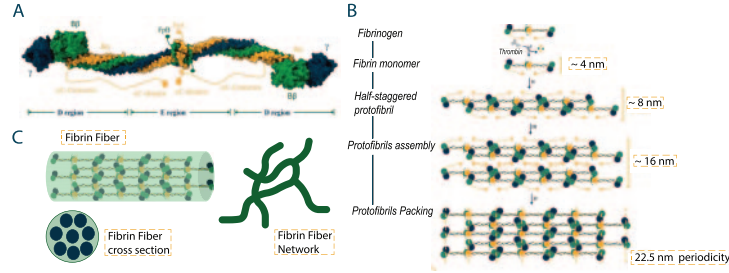


Fig. 1. – Hierarchical assembly of fibrin. (A) Fibrinogen structure (PDB ID: 1m1j) with D and E regions, Aα (orange), Bβ (green), γ (dark blue) chains, flexible αC-connectors, and fibrinopeptides FpA/B. (B) Simplified polymerization mechanism: thrombin cleavage of FpA/B forms fibrin monomers, which assemble into half-staggered protofibrils and laterally aggregate into thicker bundles. (C) Representation of the mature fiber cross-section and network architecture forming the clot scaffold.

Small-angle X-ray scattering (SAXS) is well suited to probe nanoscale fibrin organization, but until now, most studies were static and did not mimic physiological concentrations and shear [4]. Microfluidic-SAXS platforms offer controlled flow, continuous measurements, and reduced sample consumption, yet applications to protein polymerization under physiological conditions remain limited [5]. Here, we present a SAXS-compatible microfluidic device to compare fibrin polymerization under low shear (10s^{-1}) and moderate physiological shear (500s^{-1}), demonstrating how flow modulates the early nanoscale assembly in a controlled, accessible manner.

2. – Materials and methods

2.1. Microfluidic platform development. – The microfluidic device consisted of a single-inlet, single-outlet serpentine channel. Devices were fabricated by soft lithography using $500\text{ }\mu\text{m}$ thick SU-8 negative epoxy photoresist films (DJ Microlaminates, USA). The microchannels were sealed on both sides using $100\text{ }\mu\text{m}$ thick SU-8 films [5]. Channel integrity was verified by optical microscopy.

2.2. Sample preparation and polymerization. – Human fibrinogen (Sigma-Aldrich, F3879) was prepared in 10 mM Tris-HCl buffer (pH 7.4) with 150 mM NaCl. Human alpha-thrombin (Prolytix, HCT-0020) was prepared in BTC buffer (5 mM Bis-Tris, 5 mM Tris, 10 mM CHES, pH 8). For SAXS, fibrinogen was diluted to 4 mg/mL in clotting buffer (BTC, pH 7.4, 150 mM NaCl). Polymerization was initiated by adding thrombin to a final concentration of 0.45 U/mL just before the experiment.

Experiments were conducted under two flow regimes: lower shear in a flow-through capillary ($\dot{\gamma} \approx 10\text{ s}^{-1}$) and higher shear in a microfluidic device ($\dot{\gamma} \approx 500\text{ s}^{-1}$, corresponding to an estimated wall shear stress of $\tau \approx 8.5\text{ dyn/cm}^2$), all at room temperature.

2.3. SAXS data acquisition and analysis. – SAXS experiments were performed at the Austrian SAXS beamline (Elettra Synchrotron, Trieste) [6] to monitor fibrin polymerization under different shear conditions. The x-ray energy was 8.0 keV ($\lambda = 1.54\text{ }\text{\AA}$), and sample-to-detector distances were chosen to cover a q -range of $0.01\text{--}0.5\text{ }\text{\AA}^{-1}$.

Flow-through capillary setup: a 1.5 mm quartz capillary was used. The reacting solution was loaded into a syringe immediately after thrombin addition and pumped

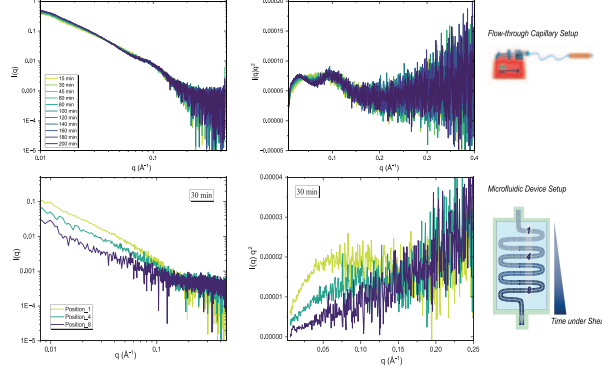


Fig. 2. – Spatiotemporal characterization of fibrin assembly under flow. Top panels: SAXS intensity profiles $I(q)$ and corresponding Kratky plots $I(q)q^2$ recorded over 200 min in a flow-through capillary under low shear (10 s^{-1}). Bottom panels: SAXS profiles and Kratky plots measured at different positions along the serpentine microchannel (Positions 1, 4, and 8), corresponding to increasing time under shear (500 s^{-1}). The side schemes illustrate the experimental setup corresponding to the data.

through the capillary at 10 s^{-1} , hence three consecutive 10 s measurements were collected every 20 min over 200 min.

Microfluidic device setup: The microfluidic device was mounted in a custom holder and connected to a programmable syringe pump. SAXS measurements were performed at 8 positions along the central channel every 30 min over 300 min, with three consecutive 10 s exposures at each position to track fibrin structural evolution as a function of time and shear.

Data reduction, including normalization, transmission correction, and background subtraction, was performed using Igor Pro.

3. – Results and discussion

3.1. Low-shear polymerization. – Fibrin polymerization under lower-shear conditions ($\dot{\gamma} \approx 10 \text{ s}^{-1}$, flow-through capillary) served as a quasi-static reference. Time-resolved profiles show a progressive increase in low- q scattering, reflecting the growth of fibrin aggregates (top of fig. 2). Simultaneously, a broad shoulder appears in the intermediate- q regime ($0.08 \text{ \AA}^{-1}$ to $0.16 \text{ \AA}^{-1}$), which should correspond to lateral protofibril packing within the fibers [4]. This feature intensifies and stabilizes in the late stages, consistent with the hierarchical fiber assembly (fig. 1(B)).

Kratky plots ($I(q)q^2$ vs. q) support this interpretation: early profiles show broad, low-amplitude features indicative of globular flexible species, while later profiles develop very pronounced peaks, signaling the transition to compact, multi-domain fibrin fibers. These SAXS signatures prove that, under low shear, fibrin assembles via ordered protofibril growth and lateral bundling into structured fibers.

3.2. Microfluidic moderate-shear polymerization. – Fibrin polymerization was induced under controlled higher shear conditions ($\dot{\gamma} \approx 500 \text{ s}^{-1}$) using the SAXS-compatible microfluidic device, and three positions (P1, P4, P8) along the serpentine channel (fig. 2) were investigated.

Representative SAXS data after 30 min reveal clear shear-dependent differences. Upstream samples (P1) exhibit compact Kratky peaks, consistent with low-shear polymerization. Downstream positions (P4 and P8) show progressively broadened, less-defined profiles, consistent with more disordered, perhaps partially unfolded, aggregates. Pronounced low- q upturns indicate the formation of larger, heterogeneous aggregates rather than organized fibers. Notably, the intermediate- q shoulder associated with lateral protofibril packing is reduced or absent, demonstrating that shear redirects fibrin assembly to heterogeneous structures, as recently demonstrated for insulin [7].

4. – Conclusions

We demonstrate that a SAXS-compatible microfluidic platform enables *in situ* investigation of fibrin polymerization under controlled shear. Low shear (10s^{-1}) supports canonical hierarchical assembly into ordered fibers, whereas moderate physiological shear (500s^{-1}) disrupts lateral protofibril packing, leading to heterogeneous aggregates and suppression of intermediate- q signatures of ordered structures. These results show that even shear rates that overlap with those found in venous and arterial blood flow can significantly influence fibrin assembly at the nanoscale, highlighting the physiological relevance of hydrodynamic forces in early clot formation [8]. Inherited and acquired fibrinogen modifications are known to systematically alter clot architecture and fibrinolytic susceptibility in cardiovascular and metabolic diseases [9]. Recent cryo-EM and integrative structural studies have made coagulation factor intermediates structurally tractable, dissecting the contributions of the mechanosensitive domain to fiber formation [10, 11]. Our results suggest hemodynamic forces constitute an additional axis of fibrin structural regulation: pathological flow disturbances may imprint aberrant nanoscale fibrin architecture independently of biochemical dysregulation. Microfluidic SAXS thus offers both a foundation for future mechanistic studies of the coagulation cascade and a potential platform for screening structure-modifying therapeutic interventions.

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