Supporting information to

Nanomechanical clinical coagulation diagnostics and monitoring of therapies

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Coagulation times (aPTT and PT) of commercially available plasma samples

To calibrate the device we have tested three different plasma samples (normal, low abnormal and high abnormal plasma controls) that have a specific aPTT or PT when tested with Werfen devices. These coagulation times are provided on the datasheet of the specific reagent used. For aPTT tests we used HemosIL APTT-SP (Werfen, 0020006300) while for PT tests we used HemosIL RecombiPlasTin 2G (Werfen, 0020003050). **Table S1** reports the coagulation times that can be found on the reagents datasheet. Depending on what device is used the values spans over a narrow range.

	aPTT (s)	PT (s)
Normal Control	28.0 – 29.3	10.3 – 12.5
Low Abnormal Control	42.4 – 46.2	19.9 – 23.8
High Abnormal Control	56.3 – 72.2	30.4 – 36

Table S1 – Activated partial thromboplastin time (aPTT) and prothrombin time (PT) as reported on the specific reagent datasheet (HemosIL APTT-SP, Werfen, 0020006300 for aPTT and HemosIL RecombiPlasTin 2G, Werfen, 0020003050 for PT). Datasheets are available online on the manufacturer website.

Determination of prothrombin time

The prothrombin time (PT) for three different plasma samples (normal, low abnormal and high abnormal) was calculated following the same procedure of activated partial thromboplastin time (aPTT). A newly developed mathematical model was fitted to the viscosity data (see **Fig. S1**) and the parameter t_0 is equal to the expected PT (see main text). For clarity we report here the equation that describes the mathematical model employed (see main text):

$$\eta(t) = A + \frac{B}{1 + e^{k(t-t_0)} + e^{c(t-t_1)}} \quad (1)$$

where $\eta(t)$ is plasma viscosity changing during coagulation and A, B, t_0, t_1, k, c are fitting parameters.

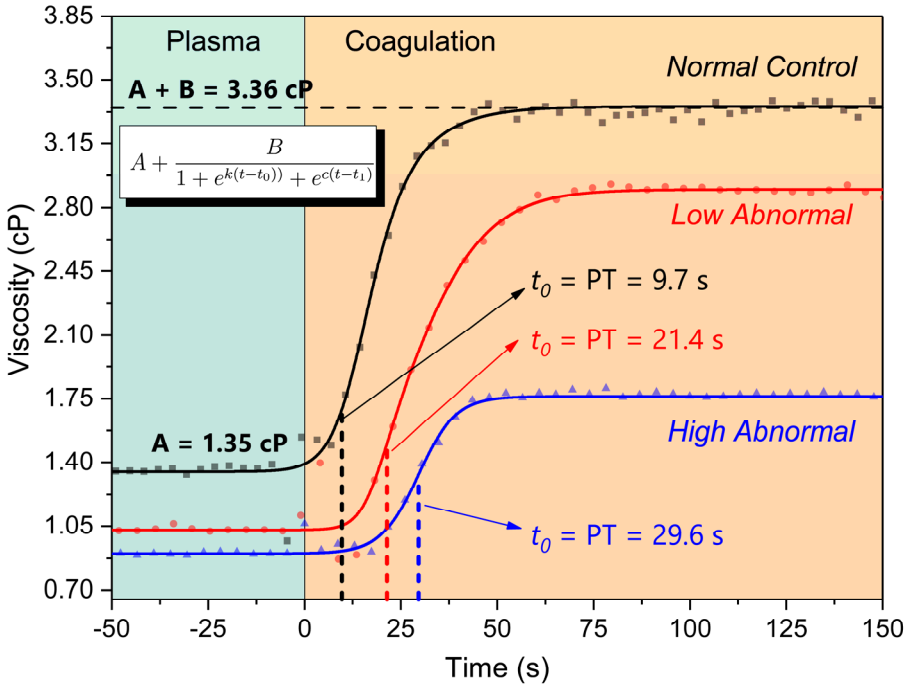

Figure S1 – Viscosity-time plots for three different plasma samples (normal control, black curve; low abnormal control, red curve; high abnormal control, blue curve). Coagulation was triggered with prothrombin time (PT) reagents. The equation in the white square was fitted to data and the extracted parameter t_0 is the prothrombin time.

Recovery of sensors and results repeatability

The array of sensors can be re-used after an automatic, *in-situ* regeneration procedure. The process includes incubation of the sensors chip in a pepsin/hydrochloric acid cleaning solution for 30 minutes. The cleaning agent is then flushed out of the chamber with a thorough rinse of 1 mM HEPES buffer. Both resonance frequency and quality factors were restored back to starting values (data not shown) and the coagulation test was repeated. Plasma proteins are considered to be stable for 4 hours after reconstitution. The starting viscosity level after 2 ½ hours was therefore lower than freshly reconstituted plasma, but the aPTT was still in the normal range (~30 s) even after a long period (see **Fig. S2**). Due to this loss of clot strength and protein activity over time every experiment presented in this work was conducted after 1 hour from reconstitution to ensure results comparability and repeatability.

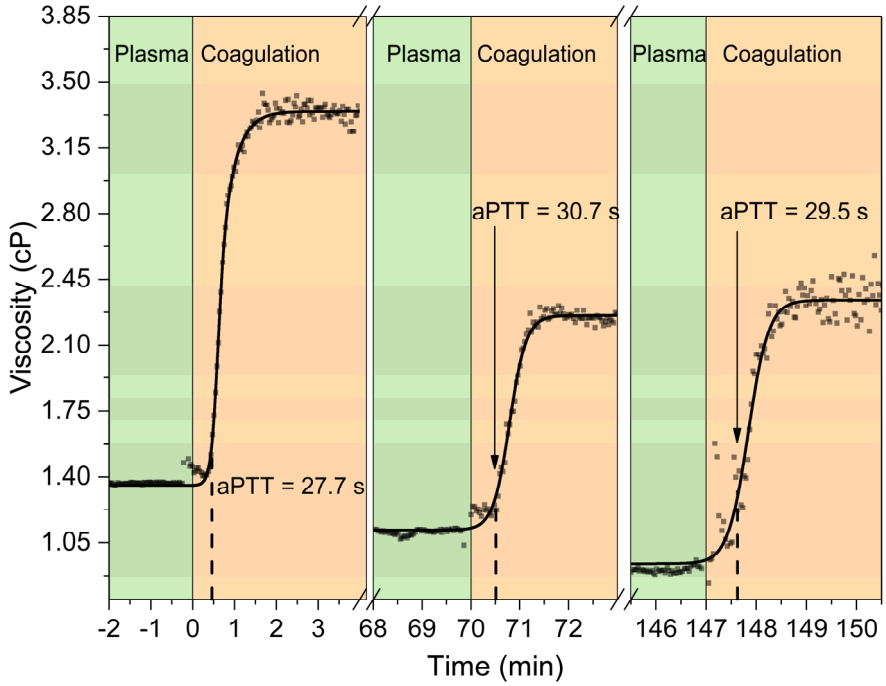

Figure S2 – Viscosity-time plots for prothrombin time test using normal control plasma. The array of sensors was first immersed in normal control plasma (green area) and then at time 0 min the coagulation was triggered with aPTT reagents and the viscosity was recorded for another 10 minutes (orange area). After 10 minutes the microfluidic chamber was flushed with pepsin/hydrochloric acid (pepsin/HCl) cleaning solution and the array of sensors was incubated for 30 minutes in this cleaning agent (data not shown). The sequence plasma-coagulation cleaning was then repeated twice.

Fibrinogen polymerization with addition of factor XIII

In the main text we showed the effects of factor XIII added to a factor XIII deficient plasma. Factor XIII cross links and stabilizes the fibrin network created during coagulation. Here we show the effects of factor XIII added to a solution of thrombin and fibrinogen. Two different solutions were prepared: one solution containing 70 NIH units/ml of thrombin, and the second solution containing 2.5 mg/ml of fibrinogen and 2 IU/ml of factor XIII. The two solutions were then mixed in the microfluidic chamber and the viscosity over time was recorded (see **Fig. S3**). The clot strength was twice as high but not within the range of a normal clot control around ~3.3 cP.

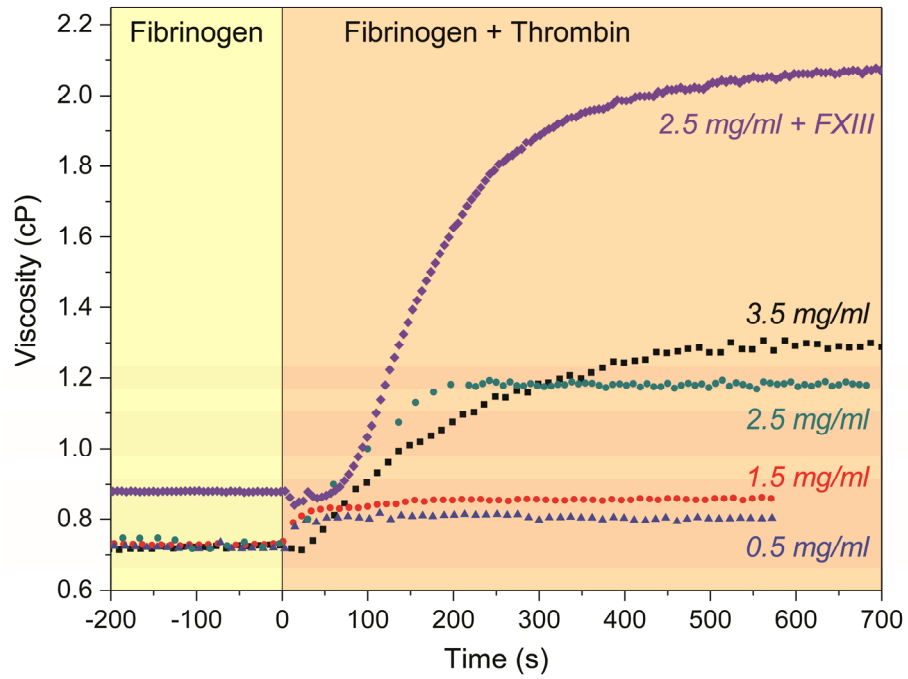

Figure S3 – Viscosity-time plots for thrombin and fibrinogen mixture solutions. The effects of factor XIII added to the mixture were tested against different fibrinogen concentrations. After incubating the array of sensors in pure fibrinogen solution (yellow area) the polymerization was triggered (at time 0 s) with addition of 70 NIH units/ml of thrombin solution (orange area).