Supporting Information

Buffers strongly modulate fibrin self-assembly into fibrous networks

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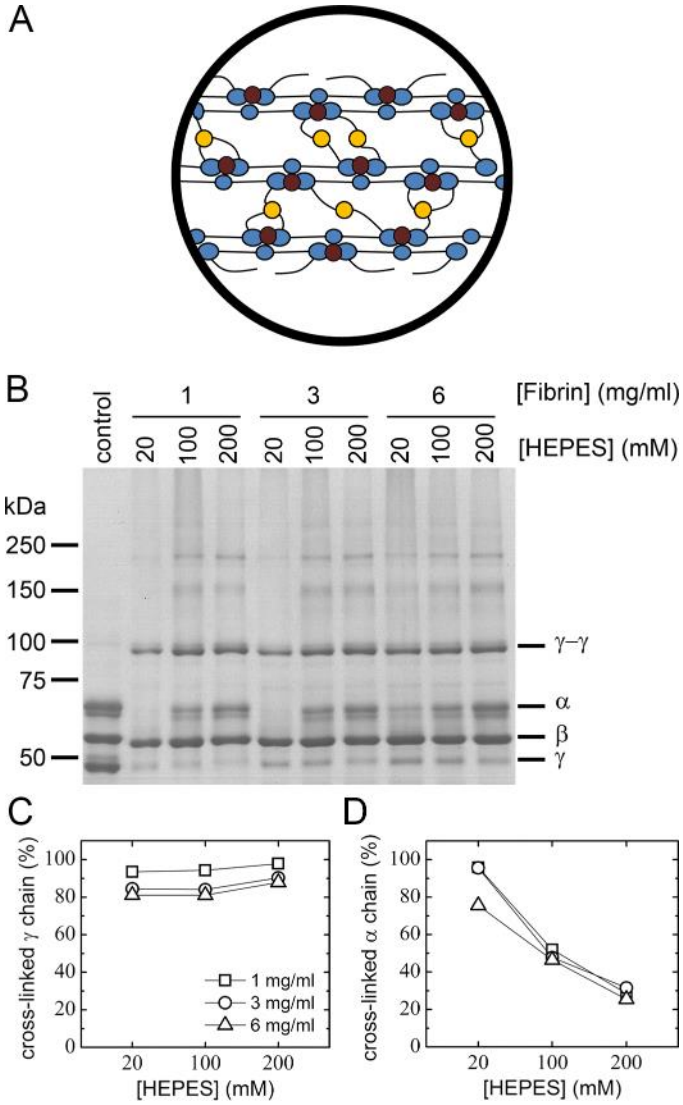

Figure S1. Factor XIII-mediated cross-linking of fibrin networks. (A) Schematic picture of part of a bundle of fibrin protofibrils, showing longitudinal γ-chain cross-links within protofibrils (*purple*) and lateral α-chain cross-links between protofibrils (*yellow*). (B) To confirm cross-linking of the networks due to endogenous Factor XIII, fibrin samples were subjected to SDS-PAGE analysis under reducing conditions. The appearance of high molecular weight bands (> 75 kDa; see marker locations on the left) and the reduction of bands corresponding to uncross-linked α- and γ-chains (see assignments on the right) confirm the formation of mature clots. The fibrin and HEPES concentrations are indicated. The control sample was a solution of fibrinogen monomer without addition of thrombin. The same amount of protein (3 μg) was loaded on all lanes. Densitometry analysis was performed to

quantify the fractions of cross-linked (C) γ-chains (within protofibrils) and (D) α-chains (between protofibrils).

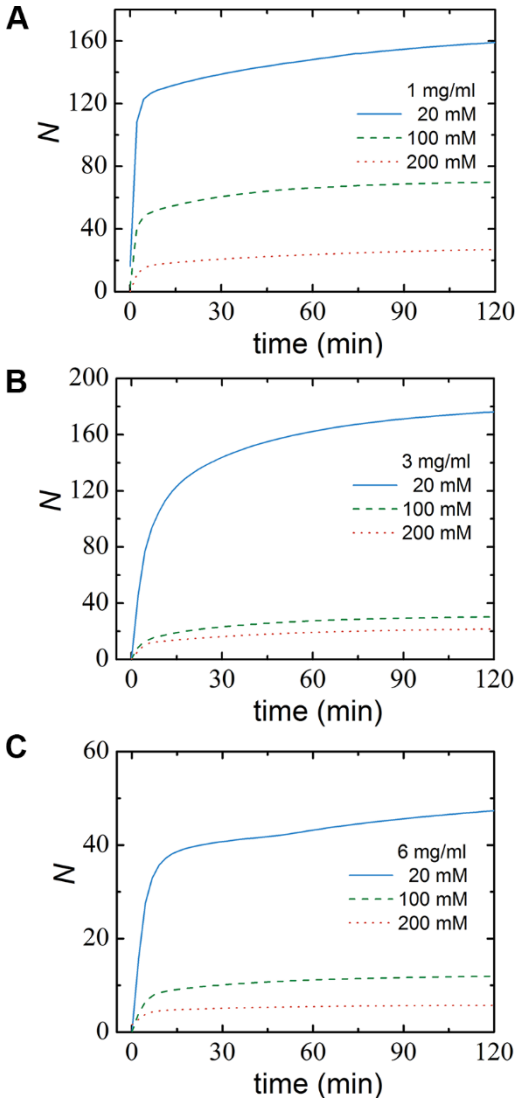

Figure S2. Turbidity measurements of the time course of protofibril lateral association in fibrin clots polymerizing at varying fibrin and HEPES concentrations. The growth of the average number of protofibrils per fiber cross-section, *N*, as the clots form was measured by analyzing the wavelength dependence of the solution turbidity and is plotted at (A) 1 mg/ml, (B) 3 mg/ml, and (C) 6 mg/ml fibrin concentrations, at 20 (*solid lines*), 100 (*dashed lines*), and 200 mM HEPES (*dotted lines*). Note that this time-resolved analysis assumes that all

fibrin monomers have been incorporated into the fibers 2.5 min after the initiation of polymerization.[1-2] Model-based interpretation of light-scattering data estimated that >70% of the monomers are already incorporated in the first 2 min,[3] suggesting that this assumption is a reasonable first approximation.

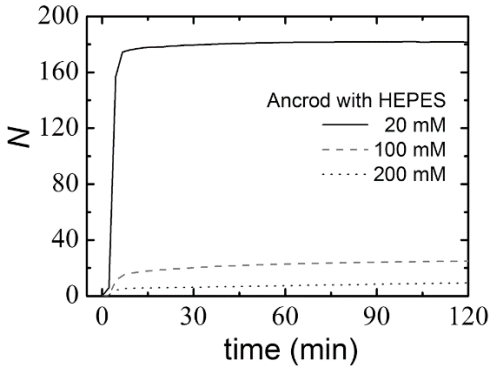

Figure S3. Turbidimetry analysis of ancrod-catalyzed fibrin polymerization at a fibrin concentration of 3 mg/ml with different HEPES concentrations. The evolution of *N*, the average number of protofibrils per fiber, is plotted as a function of time after addition of 0.5 NIH U/ml ancrod.

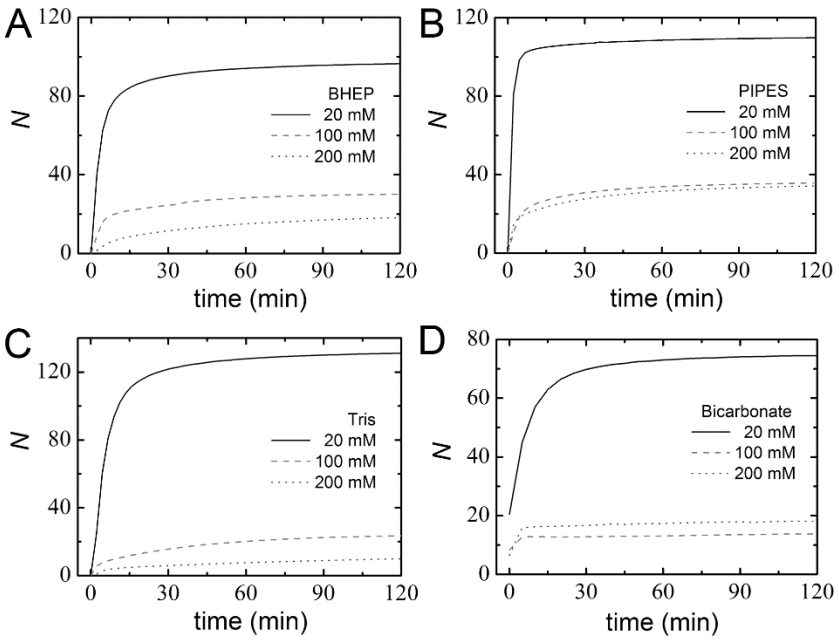

Figure S4. Turbidimetry analysis of fibrin polymerizing in various buffers. The evolution of *N* in 3 mg/ml fibrin clots formed by addition of 0.5 U/ml thrombin is evaluated at 20, 100, or 200 mM final buffer concentrations for (A) BHEP, (B) PIPES, (C) Tris, and (D) bicarbonate.

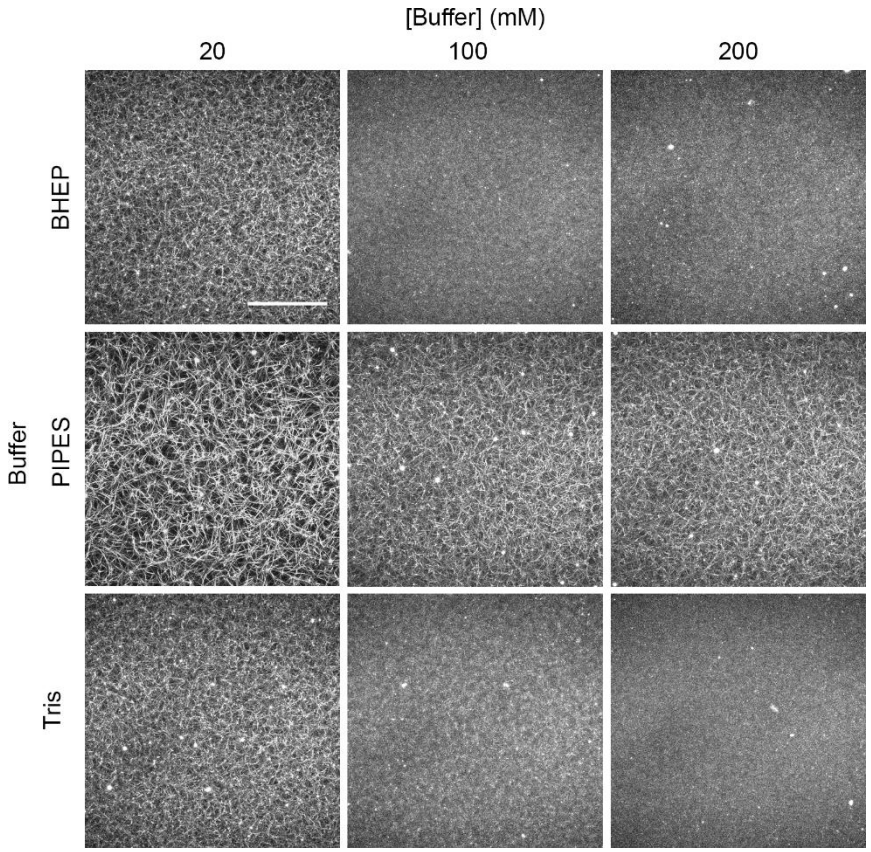

Figure S5. Confocal fluorescence images of 3 mg/ml fibrin networks formed with 20 mM, 100 mM, and 200 mM BHEP, PIPES, or Tris concentrations. Images are maximum intensity projections from z-stacks of 20 μm with 0.5 μm interval. Scale bar, 10 μm.

Table S1. Summary of reported effects of additives on fibrin structure

Type	Compound	Observed effect	Assembly condition	Proposed mechanism	Reference
pH		pH ↑ → clotting time, opacity ↓	50 mM phosphate buffer, pH 6.3–7.4, ionic strength 0.3	Possible charge effect	Ferry & Morrison[4]
ion/ionic strength	Ca2+	[Ca2+] ↑ → clotting time ↓, no effect on FpA and FpB release	Tris-imidazole buffer, ionic strength 0.18-0.24	Direct binding to fibrin(ogen)	Okada & Blömback[5]
	F-	[F-] ↑ → no change	~20 mM CHES-Tris-BisTris buffer, ionic strength 0.2	Affinity with water molecules	Di Stasio et al[6]
	Cl-	[Cl-] ↑ → turbidity, fiber diameter ↓	~20 mM CHES-Tris-BisTris buffer, ionic strength 0.2	Affinity with protein	Di Stasio et al[6]
	NaCl	ionic strength (via NaCl) ↑ → opacity goes through a minimum	50 mM phosphate buffer, ionic strength 0.15–1.5	Possible charge effect	Ferry & Morrison[4]
	NaCl	ionic strength (via NaCl) ↑ → turbidity ↓	50 mM Tris buffer, ionic strength 0.09–1.5, with 1 U/ml thrombin or 0.25 g/ml reptilase	Ca^{2+}-mediated fibrin assembly	Carr et al[7]
	NaCl	[NaCl] ↑ → turbidity ↓	20 mM phosphate buffer, pH 7.4	Kinetically-determined clot structure	Weisel & Nagaswami[8]
enzyme	thrombin	[thrombin] ↑ → clotting time ↓	Ionic strength 0.3, pH 6.64–6.81	-	Ferry & Morrison[4]
		[thrombin] ↑ → clotting time ↓	20 mM phosphate buffer, pH 7.4	Kinetically-determined clot structure	Weisel & Nagaswami[8]
		[thrombin] ↑ → fiber diameter, protofibril packing ↓	50 mM Tris buffer, pH 7.4	-	Domingues et al[9]
protein	fibronectin	[fibronectin] ↑ → turbidity ↑	40 mM Tris-imidazole buffer, ionic strength 0.15	Fibronectin acts as bridge between fibrin strands	Okada et al[10]
		[fibronectin] ↑ → turbidity ↑, fiber diameter ↓	50 mM Tris buffer	-	Ramanathan and Karuri[11]
	decorin	[decorin] ↑ → turbidity, fiber diameter ↓	20 mM HEPES buffer	Steric hindrance due to bound decorin	Dugan et al[12]
	heparin	[heparin] ↑ → fiber protein content ↓	20 mM HEPES buffer, pH 7.4	Ternary interaction between fibrin, heparin, and thrombin	Yeromonahos et al[13]
cosolvent	glycerol, glucose, propylene glycol, ethylene glycol	[reagent] ↑ → opacity ↓	pH 6.3, ionic strength 0.15	Unknown specific interaction with fibrinogen	Ferry & Morrison[4]
	starch	[starch] ↑ → opacity ↑	pH 6.3–6.7, ionic strength 0.15	Aggregation of fibrinogen	Carr[14]
	dextran	[dextran] ↑ → polymerization rate, turbidity, fiber mass-length ratio ↑	50 mM Tris buffer, pH 7.4	Dextran incorporation into fibrin fibers	Carr & Gabriel[15]
	polyphosphate	[polyphosphate] ↑ → turbidity, fiber diameter, mass-length ratio ↑	50 mM Tris buffer, pH 7.4	-	Smith & Morrissey[16]
buffer	HEPES, Tris, PIPES, BHEP	[buffer] ↑ → turbidity, fiber diameter, mass-length ratio, protofibril bundling ↓	20–200 mM buffer, pH 7.4, with 0.5 U/ml thrombin or ancrod	Slow down of protofibril lateral association	*this work*

Table S2. Conductivity and viscosity of aqueous solutions of HEPES buffer at different concentrations, but constant pH (7.4) and ionic strength (150 mM NaCl, 5 mM $CaCl_2$).

[HEPES] mM	Conductivity mS/cm	Viscosity mPa.s
20	15.7	0.71
100	16.2	0.75
200	16.5	0.78

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