Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Material: Additional Information

Integration of colloids into a semi-flexible network of fibrin

N. Ashwin K. Bharadwaj[†]*, Jin Gu Kang[‡]*, Marta H. Hatzell[‡]*,
Kenneth S. Schweizer[‡], Paul V. Braun[‡], Randy H. Ewoldt[†(a)]

[†]Department of Mechanical Science and Engineering
[‡]Department of Materials Science and Engineering
University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

1. CML size characterization (dried state)

We also use SEM imaging to characterize the size of CML particles used in our study, shown in Figure S1.

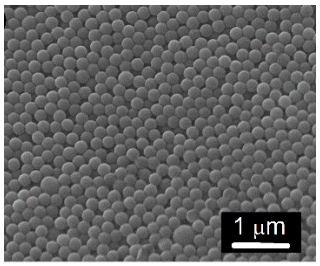


Fig S1 Dried state SEM image showing carboxylate modified latex (CML) particles with 200 nm diameter.

2. Polydispersity index (PdI) of CML in various aqueous environments

For data interpretation of DLS experiments, we used cumulant analysis based on the following equation

$$\ln\left[g^{(1)}(\tau)\right] = -\bar{\Gamma}\tau + \frac{\mu_2\tau^2}{2!} - \frac{\mu_3\tau^3}{3!} + \ldots \quad (1)$$

where $g^{(1)}(\tau)$, τ, $\bar{\Gamma}$, and μ_m indicate the first-order autocorrelation function, relaxation time, average decay rate, and variance respectively. We fit Eq. (1) to the experimental data and obtain $\bar{\Gamma}$ and μ_2, using which we define the polydispersity index (PdI) as

$$\mathrm{PdI} \equiv \frac{\mu_2}{\bar{\Gamma}^2} \quad (2)$$

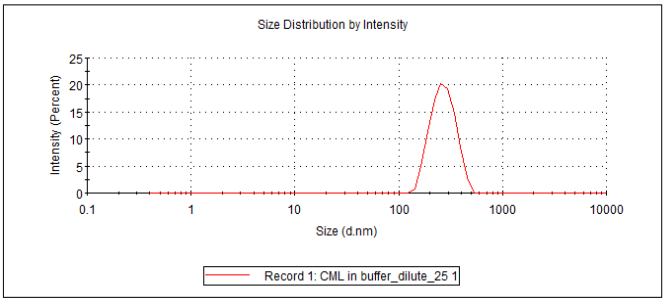
A smaller PdI is representative of monodisperse colloids in solution.

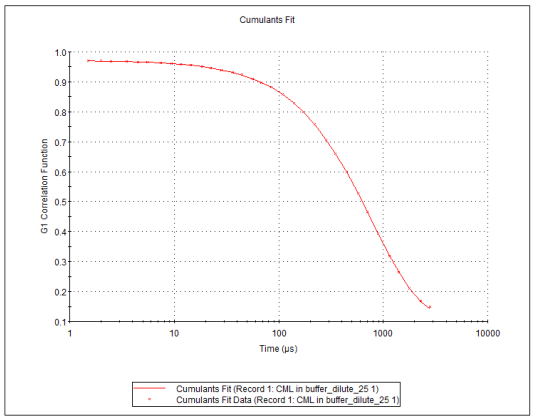
2.A. CML in buffer solution (PdI = 0.253)

Sample Name:	CML in buffer_dilute_25 1		
SOP Name:	mansettings.nano		
File Name:	CML in Buffer.dts	**Dispersant Name:**	Water
Record Number:	1	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010		

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	204.3	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	7

		Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm):	293.6	**Peak 1:**	275.0	100.0	71.58
PdI:	0.253	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.971	**Peak 3:**	0.000	0.0	0.000
Result quality :	Good				



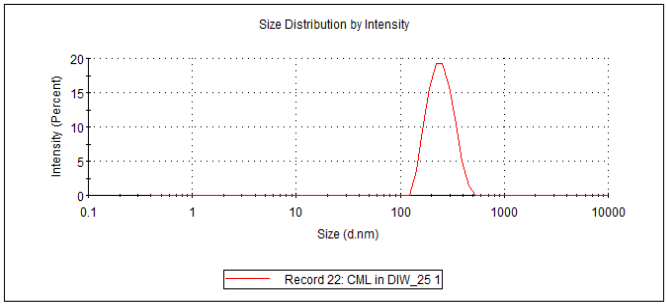


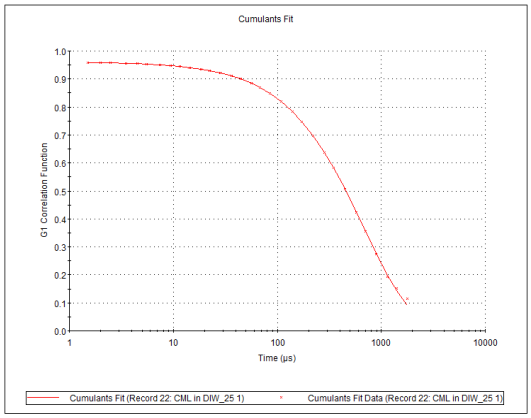
2.B CML in deionized water (PdI = 0.082)

Sample Name:	CML in DIW_25 1		
SOP Name:	mansettings.nano		
File Name:	CML in Buffer.dts	**Dispersant Name:**	Water
Record Number:	22	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010		

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	236.7	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

		Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm): 232.3	**Peak 1:**	250.7	100.0	68.82
PdI: 0.082	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.960	**Peak 3:**	0.000	0.0	0.000
Result quality : Good				



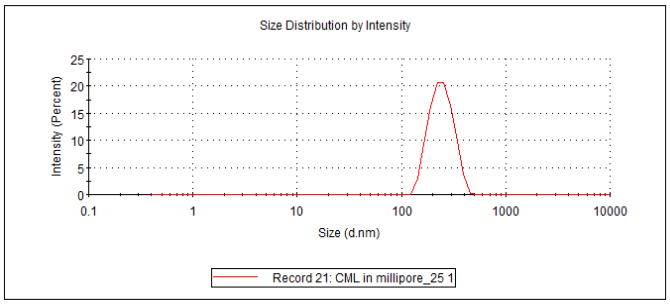


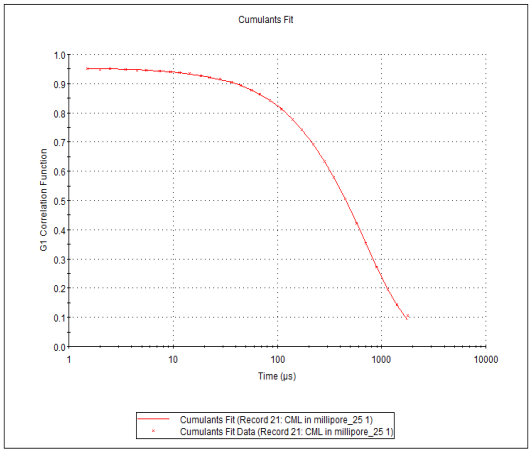
2.C. CML in Type 1 water (Millipore water: 18.2 MΩ) (PdI = 0.071)

Sample Name:	CML in millipore_25 1		
SOP Name:	mansettings.nano		
File Name:	CML in Buffer.dts	**Dispersant Name:**	Water
Record Number:	21	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010		

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	237.4	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

		Size (d.nm):	**% Intensity:**	**St Dev (d.nm):**
Z-Average (d.nm): 233.7	**Peak 1:**	246.5	100.0	62.18
PdI: 0.071	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.953	**Peak 3:**	0.000	0.0	0.000
Result quality : Good				





3. Additional confocal images to show colloid aggregation in Method 1

In addition to microstructural evidence of colloid aggregation in [F]=1.6mg/mL (Figure 4 in manuscript), we show colloid aggregation with a larger fibrinogen concentration, [F]=6.4 mg/mL, (Figure SI2).

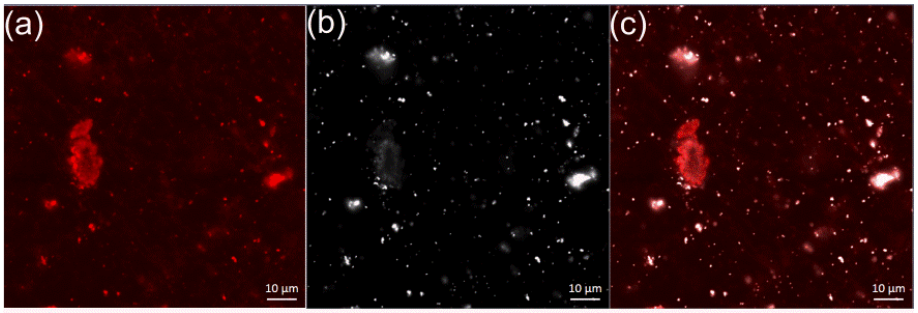
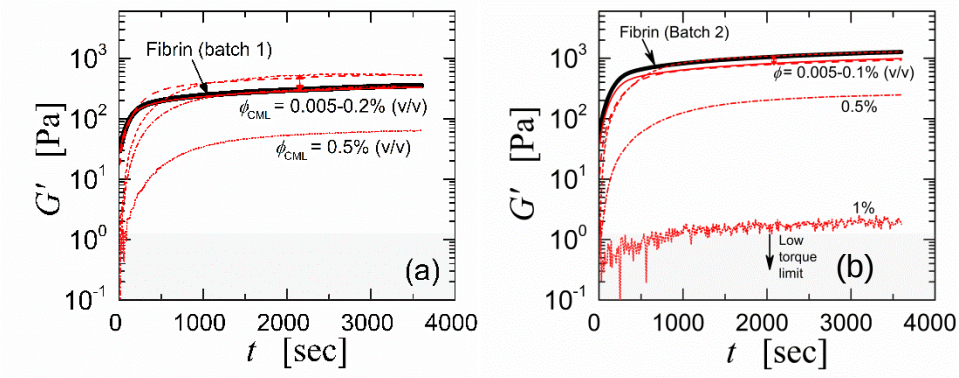


Figure S2: Confocal images showing colloid (d=200 nm) aggregation and fibrinogen recruitment for 6.4 mg/mL fibrinogen, 1.5 U/mL thrombin, and ϕ=0.05%. Signals from (a) fibrin, (b) colloids (c) fibrin+colloids.

4. Fibrinogen batch variation

We compare colloid-fibrin composites prepared using method 1 with two batches of fibrinogen (Figure 5 and Figure 8 in the manuscript). Fibrin from batch 1 has a slightly smaller elastic modulus (Figure S3(a)), compared to batch 2 (Figure S3(b)), but the ability to accommodate colloids is identical for both batches. The nonlinear properties are identical as well (Figures SI3 (c),(d)).



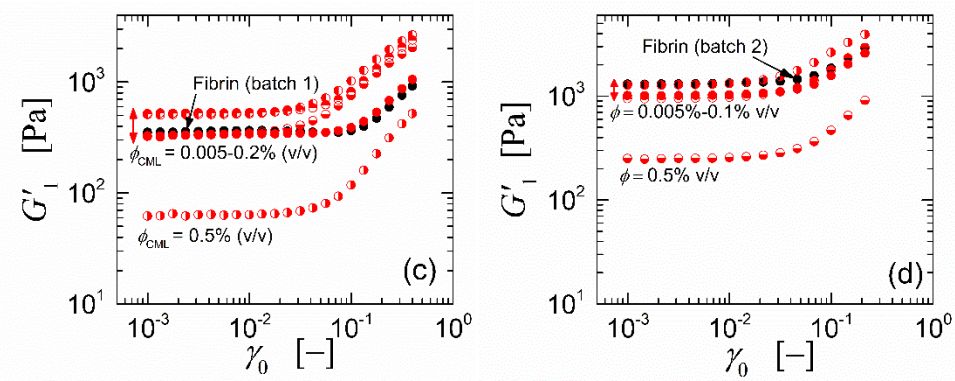


Figure S3: Fibrinogen batch variation does not affect maximum accommodable colloid volume fraction and strain stiffening behavior. Shown here for d=200 nm colloids for [F]=6.4 mg/mL and [T]=1.5 U/mL; batch 1 (a, c) and batch 2(b, d). Results from batch 2 (second row) are shown in the manuscript.

5. Evolution of elastic modulus with colloid inclusion (Method 1)

Figure 7 in the manuscript shows a phase diagram of successful/unsuccessful colloid-fibrin composites based on information from the elastic modulus of attained composites. Figures S4 (a-k) show this information for all concentrations of fibrinogen and thrombin, and colloid concentrations considered in the phase diagram.

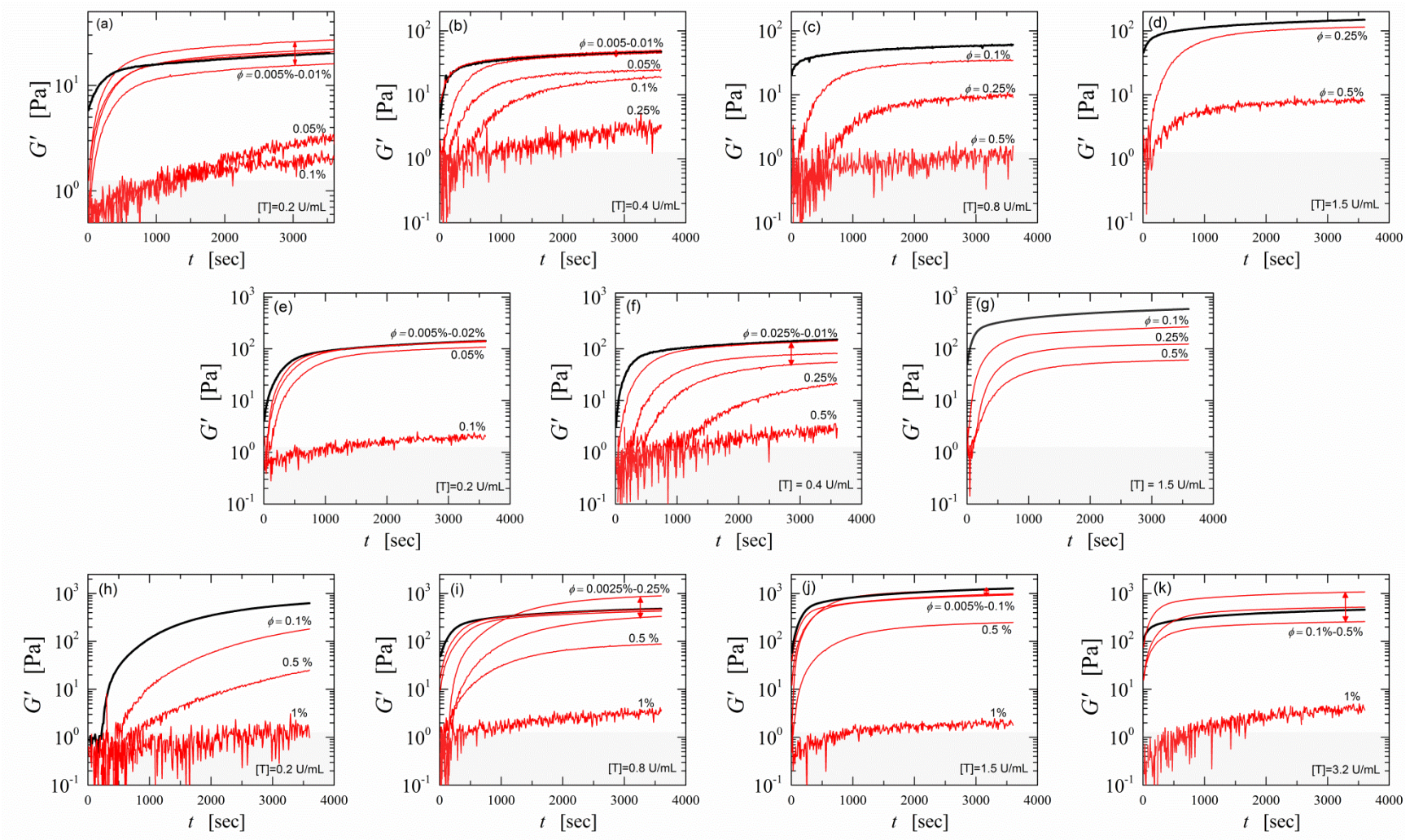


Figure S4: Evolution of elastic modulus for d=200 nm colloids in [F]=1.6 mg/mL (a-d); [F]=3.2 mg/mL (e-g); and [F]=6.4 mg/mL (h-k) with varying thrombin concentration, probed at γ_0=1% and ω=1 rad/s. In all cases, black lines with larger thickness represent neat fibrin (without colloids). Shaded light gray regions mark the instrument torque noise floor (0.5 μN.m) translating to a minimum measurable modulus of 1.25 Pa.

6. Details on elemental (C-H-N) analysis estimates of volume fraction (method 2)

Table S1 shows details in arriving at volume fractions reported in Table 1 in the manuscript. To ensure reproducibility, two samples were analyzed for each exposure time.

Table S1. Calculation of volume fraction of colloids in composites attained by electrophoretic deposition of colloids in fibrin.

t (m)	W_{dried} (mg)	C (wt%)	H (wt%)	N (wt%)	W_C (mg)	W_H (mg)	W_N (mg)	$W_{Colloids}$ (mg)	$V_{Colloids}$ (ml)	$V_{Colloids}/V_T$
1	1.1	48.93	5.73	13.14	0.5382	0.0630	0.1445	0.3569	3.399E-04	**1.700E-03**
1	1.1	48.51	6.15	13.13	0.5336	0.0677	0.1444	0.3571	3.401E-04	**1.700E-03**
3	1.3	49.01	5.81	13.29	0.6371	0.0755	0.1728	0.4206	4.006E-04	**2.003E-03**
3	1.3	49.07	5.87	13.25	0.6379	0.0763	0.1723	0.4230	4.029E-04	**2.014E-03**
5	1.5	48.66	5.89	13.66	0.7299	0.0884	0.2049	0.4719	4.494E-04	**2.247E-03**
5	1.5	48.62	6.20	13.07	0.7293	0.0930	0.1961	0.4909	4.675E-04	**2.338E-03**

(t: deposition time, W_{dried}: weight of the dried sample, W_C: total weight of carbon, W_H: total weight of hydrogen, W_N: total weight of nitrogen, $W_{Colloids}$: weight of colloids, $V_{Colloids}$: volume of colloids, V_T: total volume of the wet composite hydrogels (fixed to 0.2 ml)