SUPPLEMENTAL INFORMATION

Modular Noncovalent Functionalization of Electrospun Piezoelectric Scaffolds with Bioactive Nanocarriers

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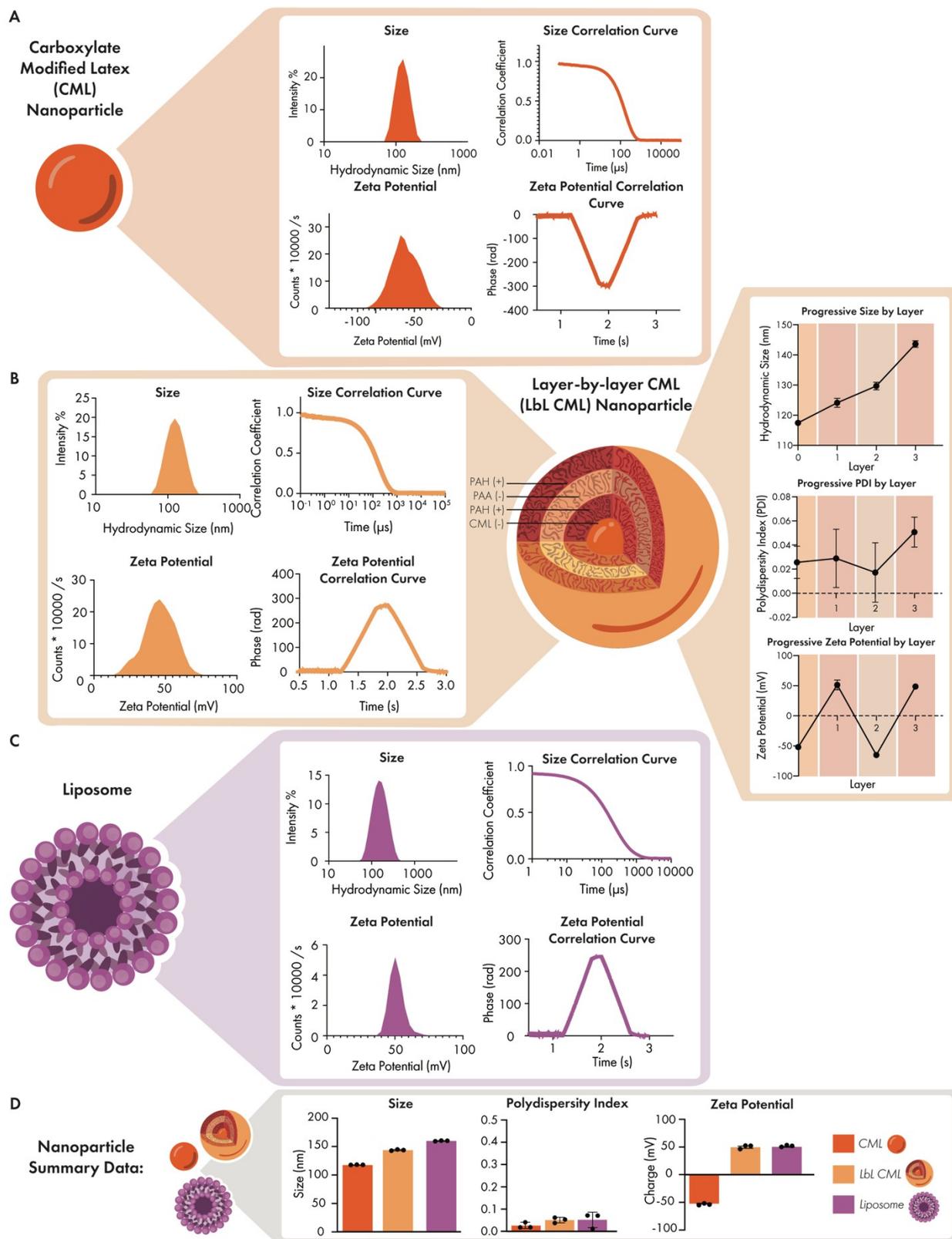
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[2] Department of Biological Sciences, Columbia University, New York, NY 10027
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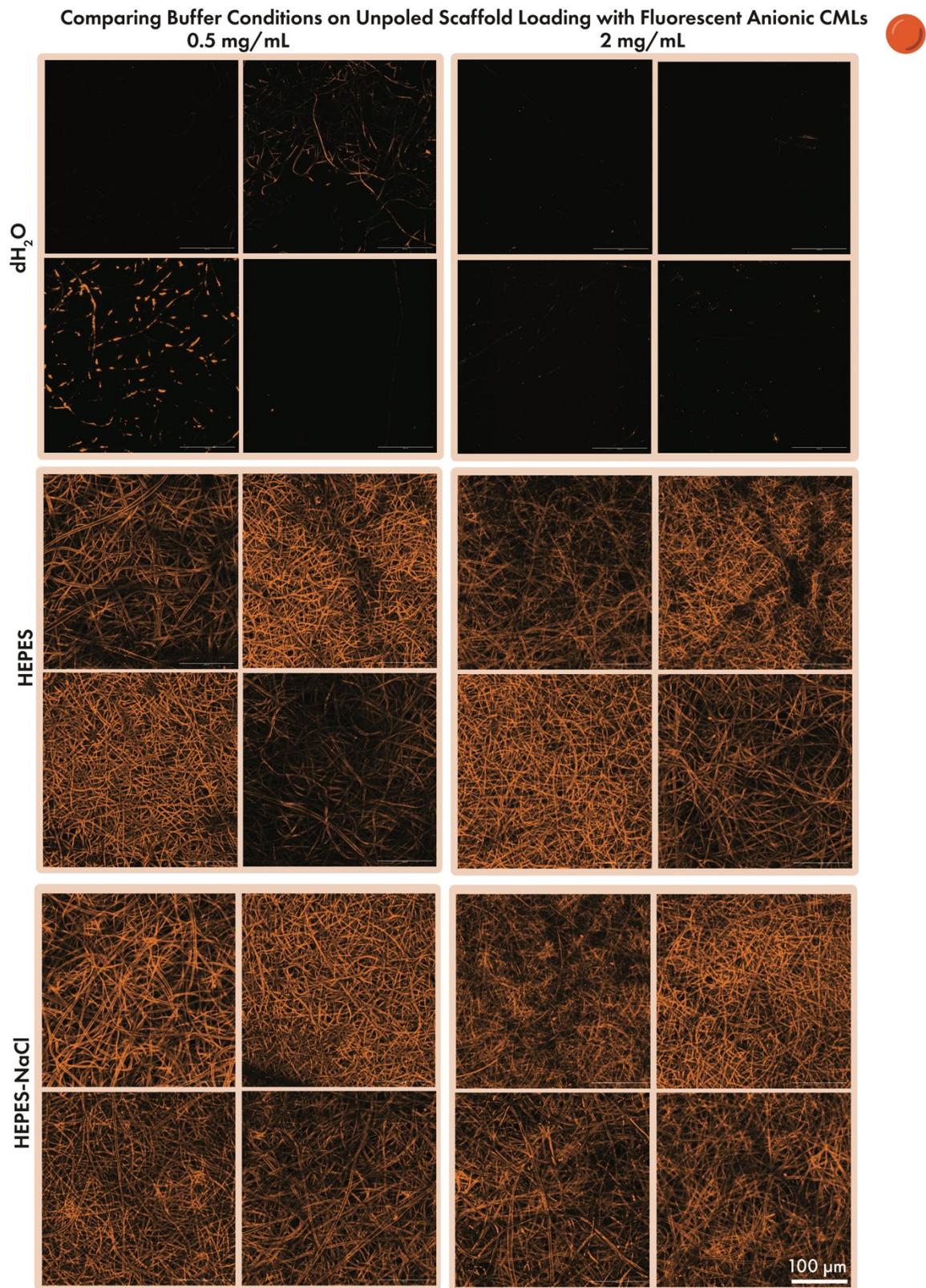[*] Correspondence: tla2132@columbia.edu ; sc5159@columbia.edu

Supplementary Figure 1: Nanoparticle summary data. Dynamic light scattering was used to characterize nanoparticle size, polydispersity index, and zeta potential. **(A)** Carboxylate modified latex (CML) particles, with size, zeta potential, and representative correlation curves. **(B)** Layer-by-layer CML

(LbL CML) with size, zeta potential, and representative correlation curves. Progressive particle characteristics (size, polydispersity index, and zeta potential) are shown with progressive coating layers (right). **(C)** Liposome size, zeta potential, and representative correlation curves. **(D)** Summary characteristics of all three particle types. Results are reported as the mean of n=3 independent syntheses. Individual mean data points are plotted blow, and results are represented as mean ± SEM.
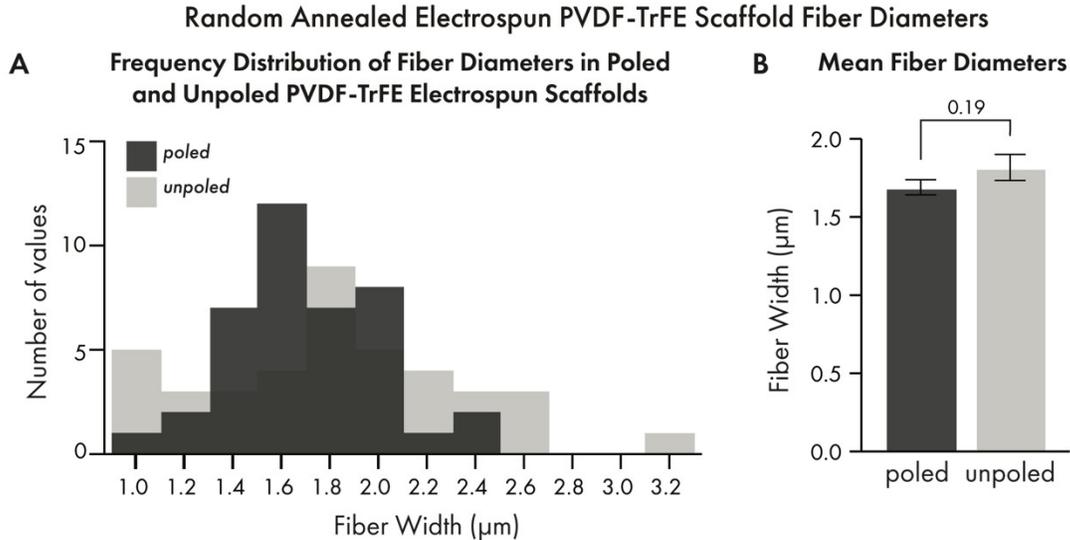
Supplementary Figure 2: Scaffold fiber size distribution. 40 fiber measurements were collected using Fiji-ImageJ from 5 independent scanning electron microscopy images of either poled or unpoled scaffolds. **(A)** Overlayed histograms of the frequency distribution are shown and **(B)** mean fiber diameters are presented. Summary result of fiber diameter is reported as mean ± SD. An unpaired t-test is used to compare the population means.

Comparing Effects of Nanoparticle Charge and Loading Buffer Conditions on Scaffold Loading, Full Images

Supplementary Figure 3: Uncropped SEM of polymeric nanoparticles on poled scaffolds (images from Figure 2), collected at 5-10K X magnification.

Supplementary Figure 4: Low-magnification SEM image of uncoated, poled scaffold (left) compared to scaffold coated in 2 mg/mL LbL CML with HEPES-NaCl (right), images acquired at 1-1.5K X magnification. Scale bars=10 μm.

Comparing Buffer Conditions on Unpoled Scaffold Loading with Fluorescent Anionic CMLs

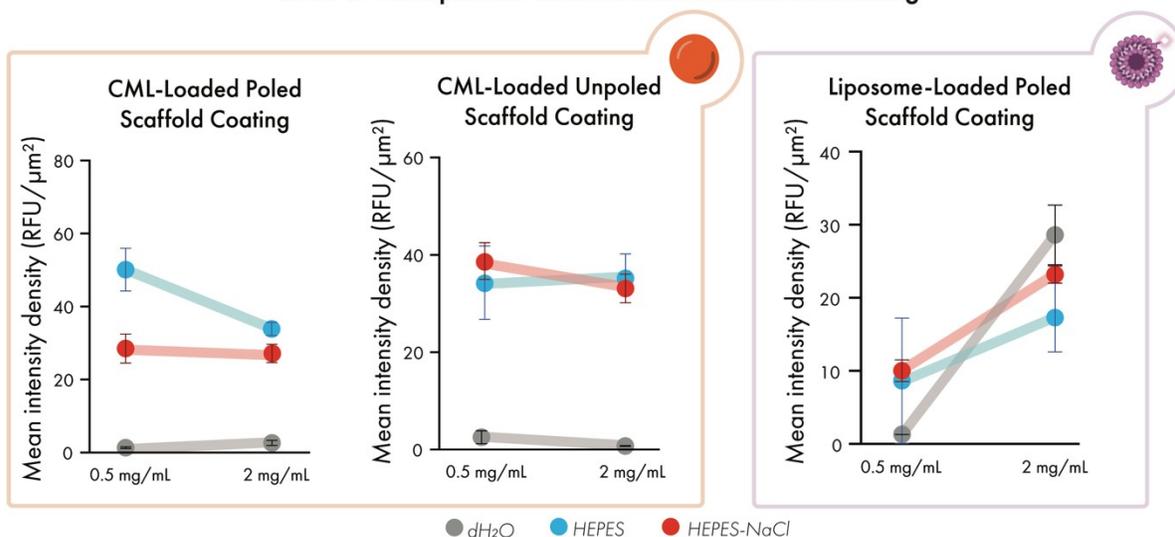**0.5 mg/mL**   **2 mg/mL**

dH₂O

HEPES

HEPES-NaCl

100 µm

Supplementary Figure 5: Anionic CML coating heterogeneity. Confocal microscopy of multiple unpoled independent scaffolds coated with fluorescent anionic CML in dH$_2$O (top), HEPES (middle), or HEPES-NaCl at low (0.5 mg/mL) and high (2 mg/mL) nanoparticle concentrations. N=4 maximum

intensity projections of 50 μm stacks were collected per condition, and the scale bar = 100 μm and is applied to all images.

Effect of Buffer Conditions on Coefficient of Variation between Particle Types and Scaffold Processings
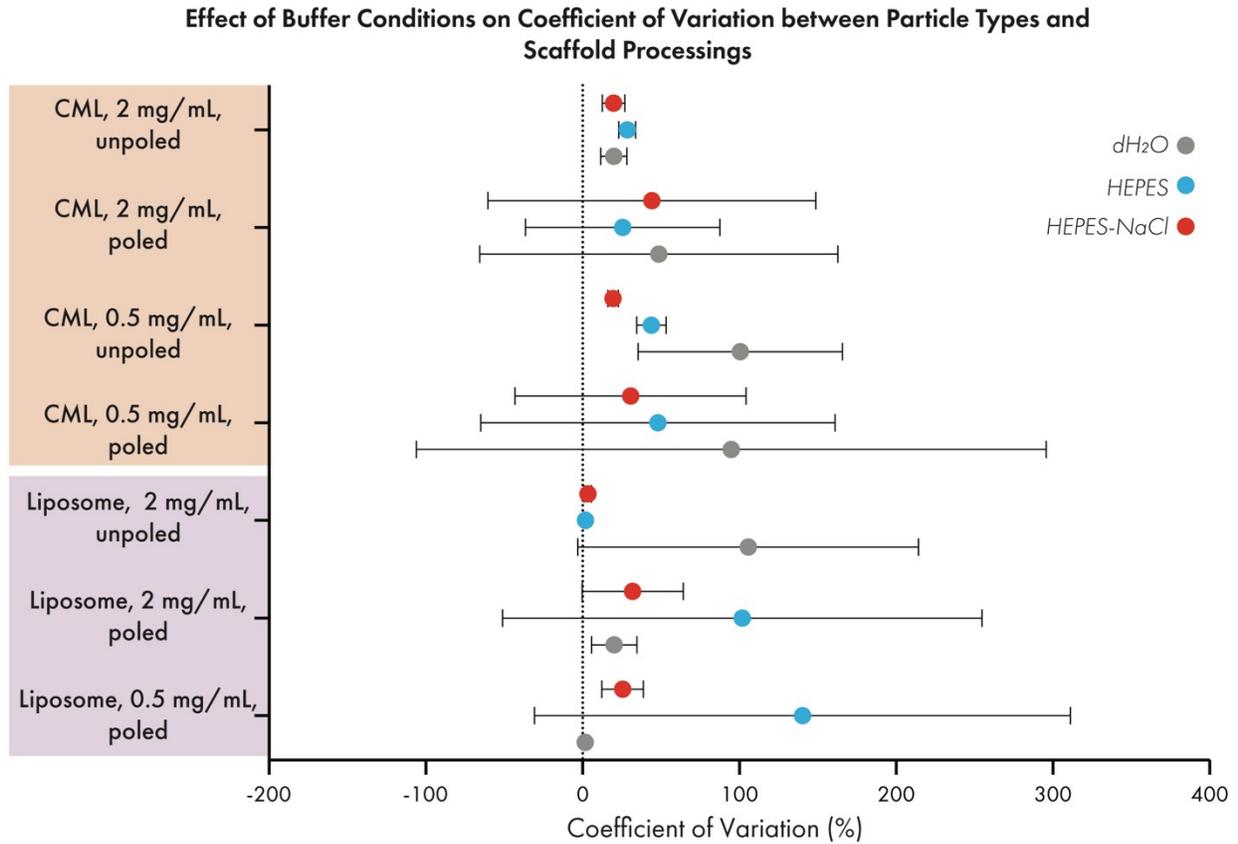
Supplementary Figure 6: Coefficients of variance between mean fluorescence intensity of coated scaffolds in different buffer conditions. Coefficients of variation by buffer type: dH₂O (grey), HEPES (blue), and HEPES-NaCl (red). Error bars represent propagated uncertainty.

Effect of Nanoparticle Concentration on Scaffold Loading

CML-Loaded Poled Scaffold Coating

CML-Loaded Unpoled Scaffold Coating

Liposome-Loaded Poled Scaffold Coating
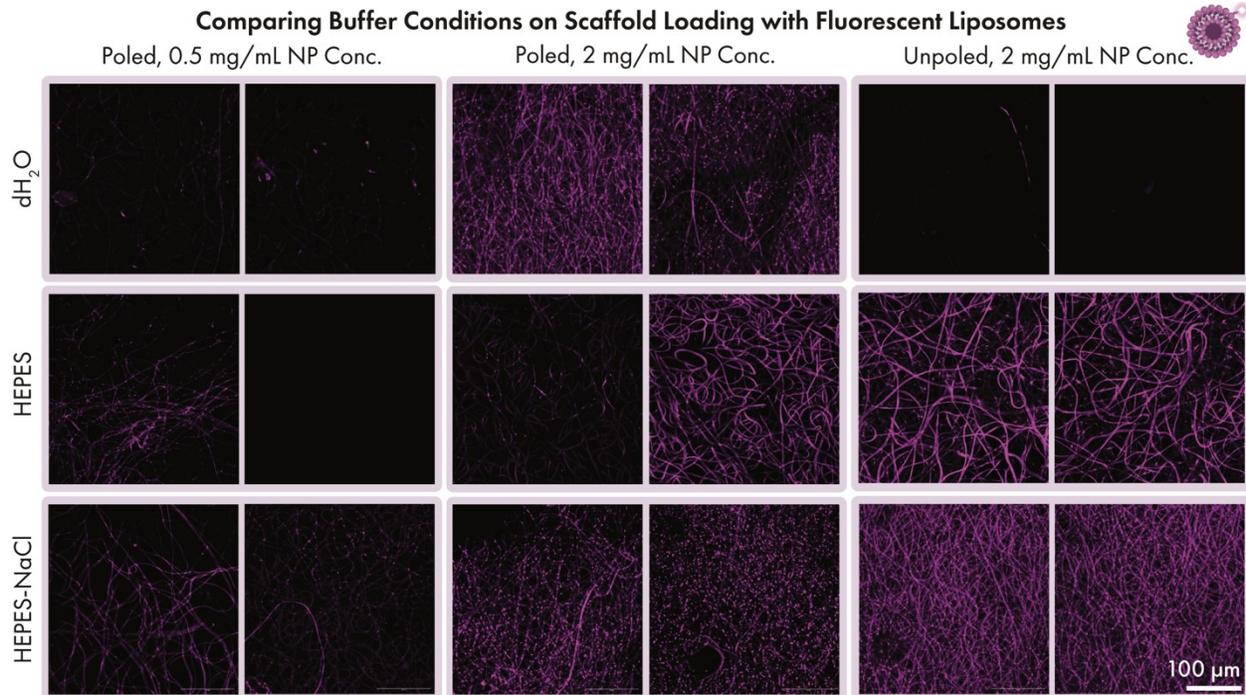
● dH₂O   ● HEPES   ● HEPES-NaCl

Supplementary Figure 7: Impact of nanoparticle concentration on scaffold loading. Scaffolds were coated at either 0.5 mg/mL or 2 mg/mL nanoparticle concentration using polymeric nanoparticles (left) or liposomes (right) in three different buffers: dH₂O (grey), HEPES (blue), and HEPES-NaCl (red). Values are represented as mean $\pm$ SEM.

Fluorescent Anionic CML Dose Response on Unpoled Scaffolds

A Representative Images of Scaffold Loading

B Scaffold Dose Response Curve
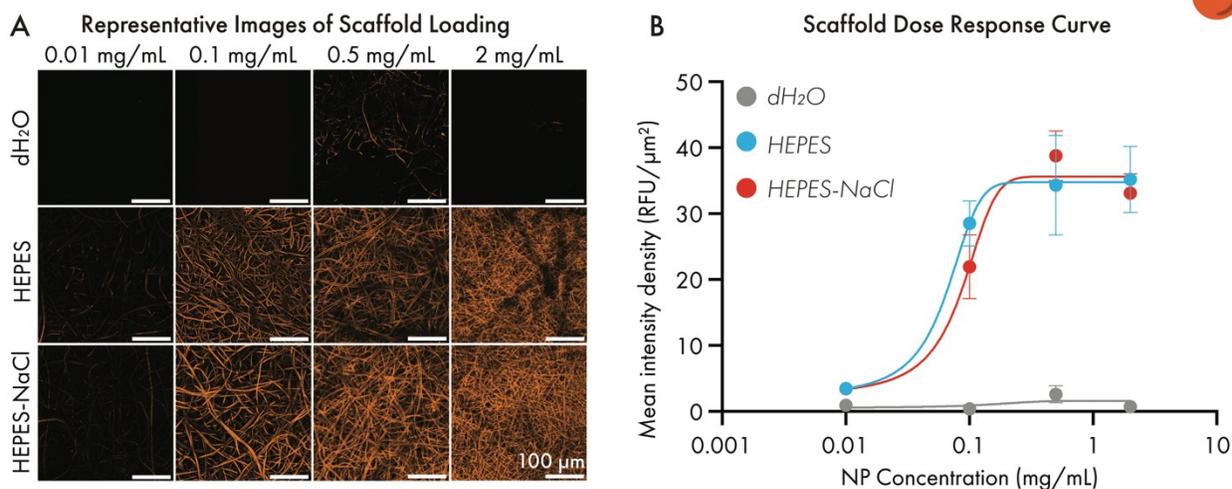
Supplementary Figure 8: Nanoparticle dose response for scaffold coating. Unpoled scaffolds were vacuum loaded with anionic CML nanoparticles. (A) representative confocal images of maximum intensity projections of 50 µm Z-stacks of nanoparticles at 0.01, 0.1, 0.5, or 2 mg/mL nanoparticle (left to right) loaded in dH2O, HEPES, or HEPES-NaCl buffer (top to bottom). (B) Dose response curve from Fiji-ImageJ quantification of four technical replicate scaffolds from conditions in (A). Scaffolds were coated at varying doses in dH₂O (grey), HEPES (blue), or HEPES-NaCl (red). Points are represented as mean $\pm$ SEM and data is fitted with nonlinear least squares fit.

Comparing Buffer Conditions on Scaffold Loading with Fluorescent Liposomes

Poled, 0.5 mg/mL NP Conc.   Poled, 2 mg/mL NP Conc.   Unpoled, 2 mg/mL NP Conc.

dH$_2$O

HEPES

HEPES-NaCl

100 µm

Supplementary Figure 9: Cationic liposome coating heterogeneity. Confocal microscopy of multiple scaffolds coated with fluorescent cationic liposomes in dH$_2$O (top), HEPES (middle), or HEPES-NaCl using poled scaffolds at low (0.5 mg/mL) density (left) and high (2 mg/mL) density (middle), or unpoled scaffolds at high density (right) nanoparticle concentrations. N=2 maximum intensity projections of 50 µm Z-stacks are shown per condition, and the scale bar = 100 µm and is applied to all images.

Piezoelectric Coefficient Decreases with Coating
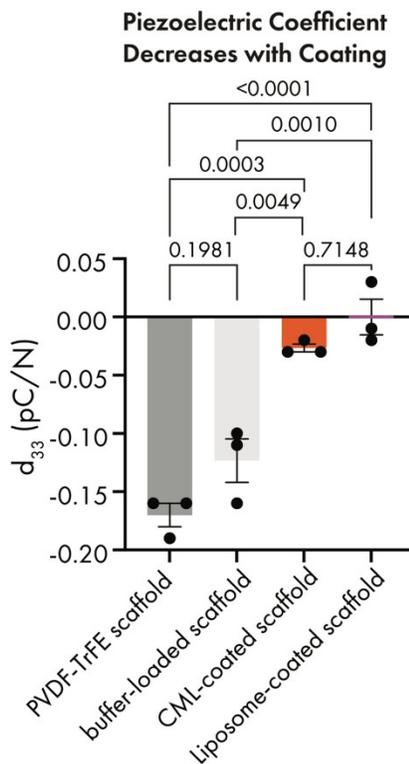
Supplementary Figure 10: Piezoelectric coefficient decreases with all particle coatings. N=3 independent unpoled scaffolds are measured. Buffer-loaded scaffold was vacuum-loaded with HEPES-NaCl, and coated scaffolds were loaded with 2 mg/mL of the corresponding particle type in HEPES-NaCl. Values are represented as mean ± SEM. Multiple comparisons following ANOVA are corrected with Šídák's multiple comparisons test.
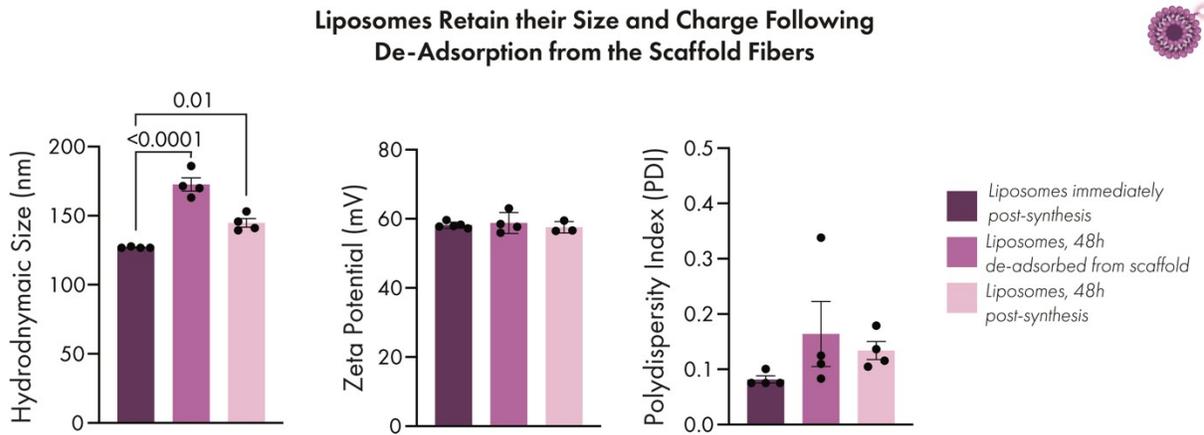
Supplementary Figure 11: Liposome characterization after de-adsorption. Dynamic light scattering measurements of hydrodynamic size (left), zeta potential (middle), and polydispersity index (right) of liposomes immediately post-synthesis (dark), 48h de-adsorbed from unpoled scaffolds (mid-tone), or 48-hours post-synthesis (light). At least n=3 technical replicates were collected per sample condition. Ordinary one-way ANOVA of size measurements is followed by a Tukey's multiple comparisons test.
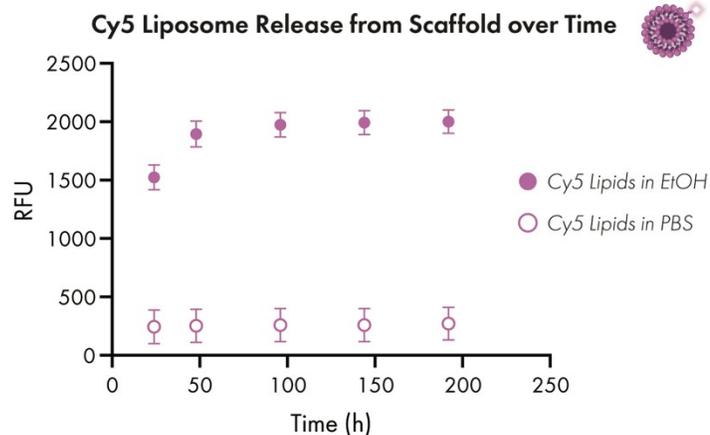
Supplementary Figure 12: Particles are retained on scaffolds in aqueous environments. Fluorometric measurements of cumulative fluorescent lipids released from unpoled scaffolds into solution over time. Subsequent data points are summations of previous release. 2 mg/mL Cy5-liposome-coated scaffolds prepared in HEPES-NaCl were incubated in 1 mL of either ethanol (closed circle) or PBS (open circle) and were shaken on a rotary shaker at 4ºC. n=3 technical replicates are measured, and values are represented as mean ± SEM.

Supplementary Table 1: Statistics

Fluorescent CML Imaging Analysis Statistics Summary, 0.5 mg/mL (Figure 3 B)										
Tukey's multiple comparisons test, a=0.05	Mean 1	Mean 2	Mean diff	SE of diff.	N1	N2	q	DF	Sum-mary	Adjusted P Value
dH2O:Poled vs. dH2O:Unpoled	1.734	2.601	-0.8668	7.931	4	4	0.1545	18	ns	>0.9999
dH2O:Poled vs. HEPES :Poled	1.734	39.59	-37.85	7.931	4	4	6.750	18	**	0.0018
dH2O:Poled vs. HEPES :Unpoled	1.734	34.33	-32.59	7.931	4	4	5.811	18	**	0.0073
dH2O:Poled vs. HEPES-NaCl:Poled	1.734	32.45	-30.72	7.931	4	4	5.477	18	*	0.0121
dH2O:Poled vs. HEPES-NaCl:Unpoled	1.734	38.76	-37.02	7.931	4	4	6.601	18	**	0.0022
dH2O:Unpoled vs. HEPES :Poled	2.601	39.59	-36.99	7.931	4	4	6.595	18	**	0.0023
dH2O:Unpoled vs. HEPES :Unpoled	2.601	34.33	-31.73	7.931	4	4	5.657	18	**	0.0092
dH2O:Unpoled vs. HEPES-NaCl:Poled	2.601	32.45	-29.85	7.931	4	4	5.322	18	*	0.0152
dH2O:Unpoled vs. HEPES-NaCl:Unpoled	2.601	38.76	-36.16	7.931	4	4	6.447	18	**	0.0028
HEPES :Poled vs. HEPES :Unpoled	39.59	34.33	5.262	7.931	4	4	0.9382	18	ns	0.9838
HEPES :Poled vs. HEPES-NaCl:Poled	39.59	32.45	7.139	7.931	4	4	1.273	18	ns	0.9415
HEPES :Poled vs. HEPES-NaCl:Unpoled	39.59	38.76	0.8323	7.931	4	4	0.1484	18	ns	>0.9999
HEPES :Unpoled vs. HEPES-NaCl:Poled	34.33	32.45	1.877	7.931	4	4	0.3346	18	ns	0.9999
HEPES :Unpoled vs. HEPES-NaCl:Unpoled	34.33	38.76	-4.430	7.931	4	4	0.7898	18	ns	0.9926
HEPES-NaCl:Poled vs. HEPES-	32.45	38.76	-6.306	7.931	4	4	1.124	18	ns	0.9648

NaCl:Unpoled									
Tukey's multiple comparisons test, a=0.05	Mean 1	Mean 2	Mean diff	SE of diff.	N 1	N 2	q	D F	Sum-mary	Adjusted P Value
dH2O:Poled vs. dH2O:Unpoled	1.721	0.7320	0.9893	5.816	4	3	0.2406	15	ns	>0.9999
dH2O:Poled vs. HEPES :Poled	1.721	30.44	-28.72	5.384	4	4	7.544	15	***	0.0010
dH2O:Poled vs. HEPES :Unpoled	1.721	30.79	-29.07	5.816	4	3	7.069	15	**	0.0018
dH2O:Poled vs. HEPES-NaCl:Poled	1.721	29.69	-27.97	5.384	4	4	7.347	15	**	0.0012
dH2O:Poled vs. HEPES-NaCl:Unpoled	1.721	36.16	-34.43	5.816	4	3	8.374	15	***	0.0003
dH2O:Unpoled vs. HEPES :Poled	0.7320	30.44	-29.71	5.816	3	4	7.225	15	**	0.0015
dH2O:Unpoled vs. HEPES :Unpoled	0.7320	30.79	-30.06	6.217	3	3	6.838	15	**	0.0025
dH2O:Unpoled vs. HEPES-NaCl:Poled	0.7320	29.69	-28.96	5.816	3	4	7.042	15	**	0.0019
dH2O:Unpoled vs. HEPES-NaCl:Unpoled	0.7320	36.16	-35.42	6.217	3	3	8.058	15	***	0.0005
HEPES :Poled vs. HEPES :Unpoled	30.44	30.79	-0.3498	5.816	4	3	0.08507	15	ns	>0.9999
HEPES :Poled vs. HEPES-NaCl:Poled	30.44	29.69	0.7503	5.384	4	4	0.1971	15	ns	>0.9999
HEPES :Poled vs. HEPES-NaCl:Unpoled	30.44	36.16	-5.714	5.816	4	3	1.390	15	ns	0.9166
HEPES :Unpoled vs.	30.79	29.69	1.100	5.816	3	4	0.2675		ns	>0.9999

								15		
HEPES-NaCl:Poled								15		
HEPES:Unpoled vs. HEPES-NaCl:Unpoled	30.79	36.16	-5.364	6.217	3	3	1.220	15	ns	0.9498
HEPES-NaCl:Poled vs. HEPES-NaCl:Unpoled	29.69	36.16	-6.464	5.816	4	3	1.572	15	ns	0.8692

Fluorescent Liposome Imaging Analysis Statistics Summary (Figure 4 B)										
Tukey's multiple comparisons test, a=0.05	Predicted (LS) mean 1	Predicted (LS) mean 2	Predicted (LS) mean diff.	SE of diff.	N 1	N 2	q	D F	Sum-mary	Adjusted P Value
dH2O:Poled vs. dH2O:Unpoled	28.67	0.3200	28.35	6.678	2	2	6.003	11	*	0.0131
dH2O:Poled vs. HEPES:Poled	28.67	17.35	11.32	5.587	2	5	2.866	11	ns	0.3868
dH2O:Poled vs. HEPES:Unpoled	28.67	34.16	-5.495	5.783	2	4	1.344	11	ns	0.9244
dH2O:Poled vs. HEPES-NaCl:Poled	28.67	23.19	5.478	6.678	2	2	1.160	11	ns	0.9575
dH2O:Poled vs. HEPES-NaCl:Unpoled	28.67	35.54	-6.872	6.678	2	2	1.455	11	ns	0.8985
dH2O:Unpoled vs. HEPES:Poled	0.3200	17.35	-17.03	5.587	2	5	4.310	11	ns	0.0890
dH2O:Unpoled vs. HEPES:Unpoled	0.3200	34.16	-33.84	5.783	2	4	8.276	11	**	0.0012
dH2O:Unpoled vs. HEPES-NaCl:Poled	0.3200	23.19	-22.87	6.678	2	2	4.843	11	*	0.0489
dH2O:Unpoled vs. HEPES-NaCl:Unpoled	0.3200	35.54	-35.22	6.678	2	2	7.459	11	**	0.0027
HEPES:Poled vs. HEPES:Unpoled	17.35	34.16	-16.82	4.480	5	4	5.309	11	*	0.0288
HEPES:Poled vs. HEPES-NaCl:Poled	17.35	23.19	-5.844	5.587	5	2	1.479	11	ns	0.8924
HEPES:Poled vs. HEPES-NaCl:Unpoled	17.35	35.54	-18.19	5.587	5	2	4.605	11	ns	0.0640
HEPES	34.16	23.19	10.97	5.783	4	2	2.683	11	ns	0.4510

	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF	Summary	Adjusted P Value
:Unpoled vs. HEPES-NaCl:Poled										
HEPES:Unpoled vs. HEPES-NaCl:Unpoled	34.16	35.54	-1.377	5.783	4	2	0.3368	11	ns	0.9999
HEPES-NaCl:Poled vs. HEPES-NaCl:Unpoled	23.19	35.54	-12.35	6.678	2	2	2.616	11	ns	0.4762
Piezoelectric Coefficient Before and After Coating (Figure 4 C)										
Tukey's multiple comparisons test, a=0.05	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF	Summary	Adjusted P Value
Uncoated PVDF-TrFE Scaffold vs. Liposome-coated, it=0	-0.1700	0.000	-0.1700	0.02285	3	3	10.52	8	***	0.0003
Uncoated PVDF-TrFE Scaffold vs. Liposome-coated, EtOH-washed, t=192h	-0.1700	-0.07000	-0.1000	0.02285	3	3	6.189	8	*	0.0101
Uncoated PVDF-TrFE Scaffold vs. Uncoated PVDF-TrFE Scaffold, EtOH washed	-0.1700	-0.1067	-0.06333	0.02285	3	3	3.919	8	ns	0.0919
Liposome-coated, t=0 vs. Liposome-coated, EtOH-washed, t=192h	0.000	-0.07000	0.07000	0.02285	3	3	4.332	8	ns	0.0608
Liposome-coated, it=0 vs. Uncoated PVDF-TrFE Scaffold, EtOH washed	0.000	-0.1067	0.1067	0.02285	3	3	6.601	8	**	0.0070
Liposome-coated, EtOH-washed, t=192h vs. Uncoated PVDF-TrFE Scaffold, EtOH washed	-0.07000	-0.1067	0.03667	0.02285	3	3	2.269	8	ns	0.4277

Effect of Liposome Coating on Live HEK Cell Count (Figure 5 A)										
Šídák's multiple comparisons test, a=0.05	Mean 1	Mean 2	Mean Diff.	SE of diff.	N 1	N 2	t	D F	Summary	Adjusted P Value
Liposome scaffold vs. uncoated scaffold, 24h	19215	11940	7275	22357	3	3	0.3254	16	ns	0.9960
Liposome scaffold vs. uncoated scaffold, 96h	87690	112148	-24458	22357	3	3	1.094	16	ns	0.7461
Liposome scaffold vs. uncoated scaffold, 144h	83581	125458	-41877	22357	3	3	1.873	16	ns	0.2819
Liposome scaffold vs. uncoated scaffold, 192h	124409	121157	3253	22357	3	3	0.1455	16	ns	0.9998
Signaling from a Scaffold (Figure 5 Dii)										
Šídák's multiple comparisons test, a=0.05	Mean 1	Mean 2	Mean Diff.	SE of diff.	n 1	n 2	t	D F	Summary	Adjusted P Value
IL-15 scaffold vs. uncoated scaffold, day 1	0.3817	0.3687	0.01300	0.04921	3	3	0.2642	16.00	ns	0.9982
IL-15 scaffold vs. uncoated scaffold, day 2	0.7040	0.7687	-0.06467	0.04921	3	3	1.314	16.00	ns	0.6052
IL-15 scaffold vs. uncoated scaffold, day 4	0.9357	0.9967	-0.06100	0.04921	3	3	1.240	16.00	ns	0.6539
IL-15 scaffold vs. uncoated scaffold, day 6	0.8617	1.084	-0.2227	0.04921	3	3	4.525	16.00	ns	0.0014
Piezoelectric Coefficient Decreases with Coating (Supplementary Figure 10)										
Šídák's multiple comparisons test, a=0.05	Mean 1	Mean 2	Mean Diff.	SE of diff.	n 1	n 2	t	D F	Summary	Adjusted P Value
PVDF-TrFE scaffold vs. buffer-loaded scaffold	-0.1700	-0.1233	-0.04667	0.01856	3	3	2.514	8	ns	0.1981
PVDF-TrFE scaffold vs. CML-coated scaffold	-0.1700	-0.02667	-0.1433	0.01856	3	3	7.723	8	***	0.0003
PVDF-TrFE scaffold vs. Liposome-coated scaffold	-0.1700	0.000	-0.1700	0.01856	3	3	9.160	8	****	<0.0001

buffer-loaded scaffold vs. CML-coated scaffold	-0.1233	-0.02667	-0.09667	0.01856	3	3	5.209	8	**	0.0049
buffer-loaded scaffold vs. Liposome-coated scaffold	-0.1233	0.000	-0.1233	0.01856	3	3	6.645	8	***	0.0010
CML-coated scaffold vs. Liposome-coated scaffold	-0.02667	0.000	-0.02667	0.01856	3	3	1.437	8	ns	0.7148
PVDF-TrFE scaffold vs. buffer-loaded scaffold	-0.1700	-0.1233	-0.04667	0.01856	3	3	2.514	8	ns	0.1981

Supplementary Table 2: Experimental Materials Table

Experiment and Figure Location	Scaffold Poling	Nanoparticle Type	Nanoparticle Concentration	Loading Mechanism	Loading Buffer
Comparison of loading methodology (Figure 1 B ,C)	Poled	Cationic LbL CMLs	2 mg/mL	Passive incubation vs. vacuum-assisted loading	dH_2O vs. HEPES vs. HEPES-NaCl
Comparing effects of nanoparticle charge and loading buffer (Figure 2)	Poled	Anionic CML vs. cationic LbL CMLs	0.5 mg/mL vs. 2 mg/mL	Vacuum-assisted loading	dH_2O vs. HEPES vs. HEPES-NaCl
Poling is not necessary for charged polymeric NP adsorption (Figure 3)	Poled vs. unpoled	Fluorescent anionic CMLs	0.5 mg/mL vs. 2 mg/mL	Vacuum-assisted loading	dH_2O vs. HEPES vs. HEPES-NaCl
Comparing scaffold coating by varying NP density, scaffold processing, and buffer conditions (Figure 4A)	Poled vs. unpoled	Fluorescent cationic liposomes	0.5 mg/mL vs. 2 mg/mL	Vacuum-assisted loading	dH_2O vs. HEPES vs. HEPES-NaCl
Effect of loading buffer and poling on scaffold coating (Figure 4B)	Poled vs. unpoled	Fluorescent cationic liposomes	2 mg/mL	Vacuum-assisted loading	dH_2O vs. HEPES vs. HEPES-NaCl
Nitrogen signal confirms liposome presence (Figure	Unpoled	Fluorescent cationic liposomes	2 mg/mL	Vacuum-assisted loading	HEPES-NaCl

4C)					
Intrinsic piezoelectric coefficient is impacted by particle coatings (Figure 4D)	Unpoled	Fluorescent cationic liposomes	2 mg/mL	Vacuum-assisted loading	HEPES-NaCl
Effect of liposome coating on live HEK cell count (Figure 5A)	Unpoled	Fluorescent cationic liposomes	2 mg/mL	Vacuum-assisted loading	HEPES-NaCl
Live Jurkat T cells infiltrate scaffolds and exhibit cytoskeletal reorganization (Figure 5B, C)	Unpoled	Fluorescent cationic liposomes	2 mg/mL	Vacuum-assisted loading	HEPES-NaCl
Surface presentation of IL-15 from scaffolds triggers signaling pathway (Figure 5D)	Unpoled	Fluorescent cationic liposomes	2 mg/mL	Vacuum-assisted loading	HEPES-NaCl