Supporting Information

Controlling Structure and Function of Polymeric Drug Delivery Nanoparticles Using Microfluidics

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Supporting Information: Critical water content data and discussion; tables of actual flow rates; complete MCF-7 antiproliferative data for PAX-loaded and empty (control) PNPs; additional TEM images.

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Critical Water Contents. Critical water contents for the three copolymers in DMF at a copolymer concentration of 0.33 wt % are listed in Table 1. These values were used to determine the on-chip water concentrations for microfluidic PNP preparations, which were set at 5.0 wt % above the critical water content for all copolymers (cwc + 5.0 wt %), so 10.5 wt % for PCL12k, 11.3 wt % for PCL6.4k and 18.1 wt % for PCL2.1k.

Table S1. Copolymer Properties and Critical Water Contents

Copolymer	$M_{n,PCL}$ / 10^3 (g mol^{-1})	$M_{n,PEO}$ / 10^3 (g mol^{-1})	f_{PCL}	PDI	cwc[a] (wt %)
PCL2.1k	2.1	5.0	0.30	1.08	13.1 ± 0.2
PCL6.4k	6.4	5.0	0.56	1.12	6.3 ± 0.2
PCL12k	12	5.0	0.71	1.12	5.5 ± 0.4

a) Determined for 0.33 wt % copolymer solutions in DMF.

Table S2. Actual Gas and Liquid Flow Rates for Various Microfluidic Preparations of PAX-loaded PCL-*b*-PEO Nanoparticles Described in Figures 2-4.

Water Content, Nominal Flow Rate	L_{gas} (x 10^3m)	L_{liq} (x 10^3m)	Q_{gas} (µL/min)	Q_{liq} (µL/min)	Q_{gas}/Q_{liq}	Q_{total} (µL/min)
PCL(2.1k)						
***Q* = 100 µL/min; *r* = 0.10**						
Prep #1	0.7	0.6	52.3	50.0	1.05	102.3
Prep #2	0.8	0.8	51.1	50.0	1.02	101.0
Prep #3	0.8	0.8	48.8	50.0	0.98	98.8
200 µL/min; *r* = 0.10						
Prep #1	0.9	0.9	105	100	1.05	205
Prep #2	1.1	1.0	111	100	1.11	211
Prep #3	1.1	1.2	94	100	0.94	194
PCL(6.4k)						
***Q* = 100 µL/min; *r* = 0.10**						
Prep #1	1.0	1.0	55	50	1.1	105
Prep #2	1.1	1.0	54	50	1.08	104
Prep #3	0.9	1.0	45	50	0.9	95
***Q* = 100 µL/min; *r* = 0.25**						
Prep #1	1.0	1.1	43.2	50	0.86	93.2
Prep #2	0.9	1.0	48.0	50	0.96	98.0
Prep #3	1.0	1	51	50	1.01	101
***Q* = 100 µL/min; *r* = 0.50**						
Prep #1	1.0	1.0	46.6	50	0.93	96.6
Prep #2	1.1	1.0	52.5	50	1.05	102.5
Prep #3	1.1	1.0	57.9	50	1.16	107.9
***Q* = 100 µL/min; *r* = 0.60**						
Prep #1	1.0	1.1	45	50.0	0.90	95
Prep #2	1.0	1.0	50.8	50.0	1.01	100.8
Prep #3	1.1	1.0	56.1	50.0	1.12	106.1
***Q* = 200 µL/min; *r* = 0.1**						
Prep #1	1.2	1.0	120	100	1.2	220
Prep #2	1.1	0.9	122	100	1.2	222
Prep #3	0.9	0.9	103	100	1.03	203
PCL(12k)						
***Q* = 100 µL/min; *r* = 0.10**						
Prep #1	0.9	1.0	46.5	50	0.93	96.5
Prep #2	0.9	0.9	52.2	50.0	1.04	102
Prep #3	1.0	0.9	57	50	1.13	107

***Q* = 200 µL/min; *r* = 0.10**						
Prep #1	1.0	1.0	98	100	0.98	198
Prep #2	0.95	0.9	106	100	1.06	206
Prep #3	1.0	1.1	95	100	0.95	195

Table S3. Actual Gas and Liquid Flow Rates for Various Microfluidic Preparations of PAX-Loaded PCL-*b*-PEO Nanoparticles Described in Figures 5 and 6.

Nominal Flow Rate[a]	L_{gas} **(x 10^3m)**	L_{liq} **(x 10^3m)**	Q_{gas} **(µL/min)**	Q_{liq} **(µL/min)**	Q_{gas}/Q_{liq}	Q_{total} **(µL/min)**
PCL(6.4k)						
***Q* = 100 µL/min; *r* = 0.60**						
Prep #1	1.0	1.1	45	50.0	0.90	95
Prep #2	1.0	1.0	50.8	50.0	1.01	100.8
Prep #3	1.1	1.0	56.1	50.0	1.12	106.1
***Q* = 200 µL/min; *r* = 0.60**						
Prep #1	1.0	1.0	98	100	0.98	198
Prep #2	1.01	1.1	96	100	0.96	196
Prep #3	0.95	0.9	106	100	1.06	206
***Q* = 400 µL/min; *r* = 0.60**						
Prep #1	1.0	1.0	194	200	0.97	394
Prep #2	1.1	0.9	233	200	1.2	433
Prep #3	1.1	1	220	200	1.1	420

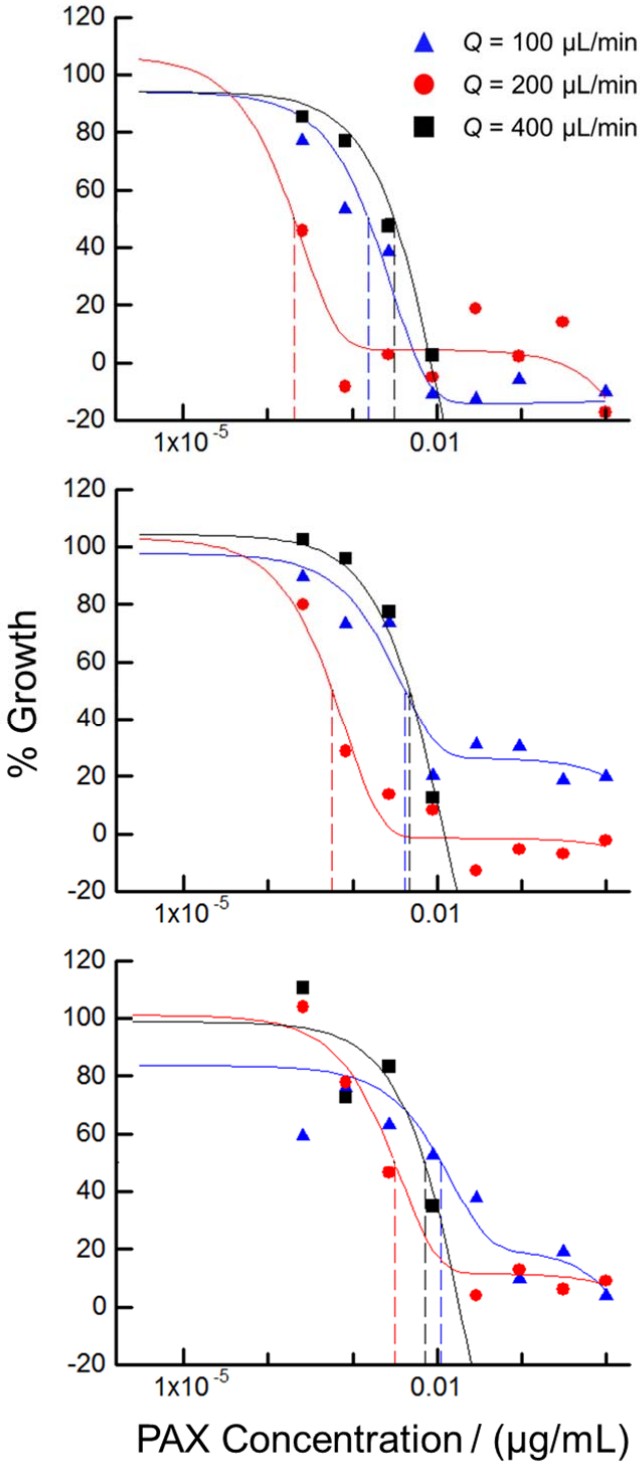

Figure S1. Dependence of flow rate (Q = 100, 200, and 400 µL / min) on growth inhibition plots for microfluidic-prepared PNPs: (A) 48-h, (B) 72-h and (C) 96-h. Solid lines represent best fit curves and dashed vertical lines indicate GI_{50} values. Data points represent mean data from triplicate PNP preparations under each condition.

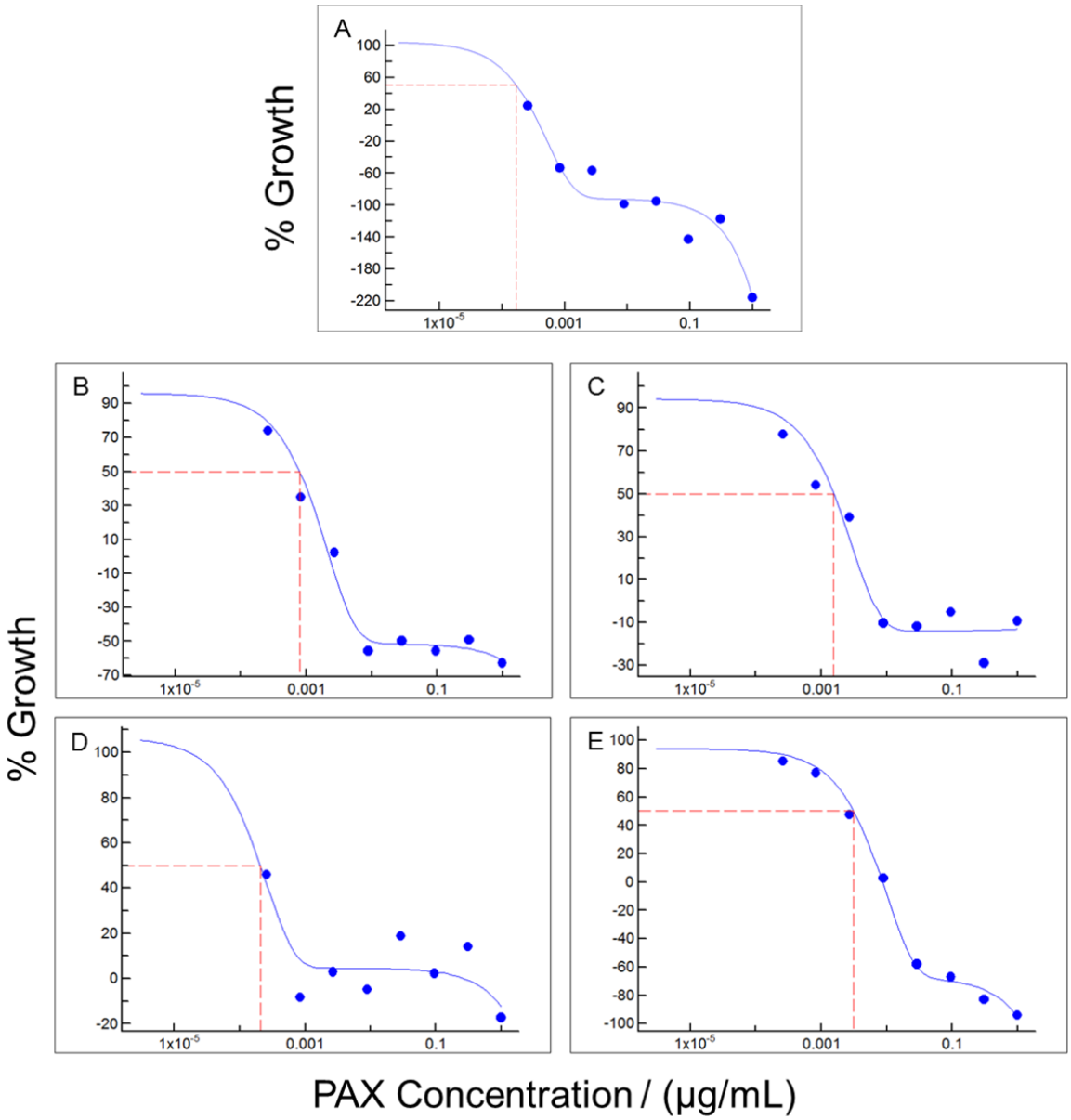

Figure S2. 48 h-Growth inhibition plots for (A) free PAX, (B) bulk-prepared PAX-loaded PNPs, and PAX-loaded PNPs prepared in the microfluidic reactor at different flow rates: (C) Q = 100 µL / min, (D) Q = 200 µL / min, and (E) Q = 400 µL / min. Data points in B-E represent mean data from triplicate PNP preparations under each condition. Dashed red lines indicate GI_{50} values.

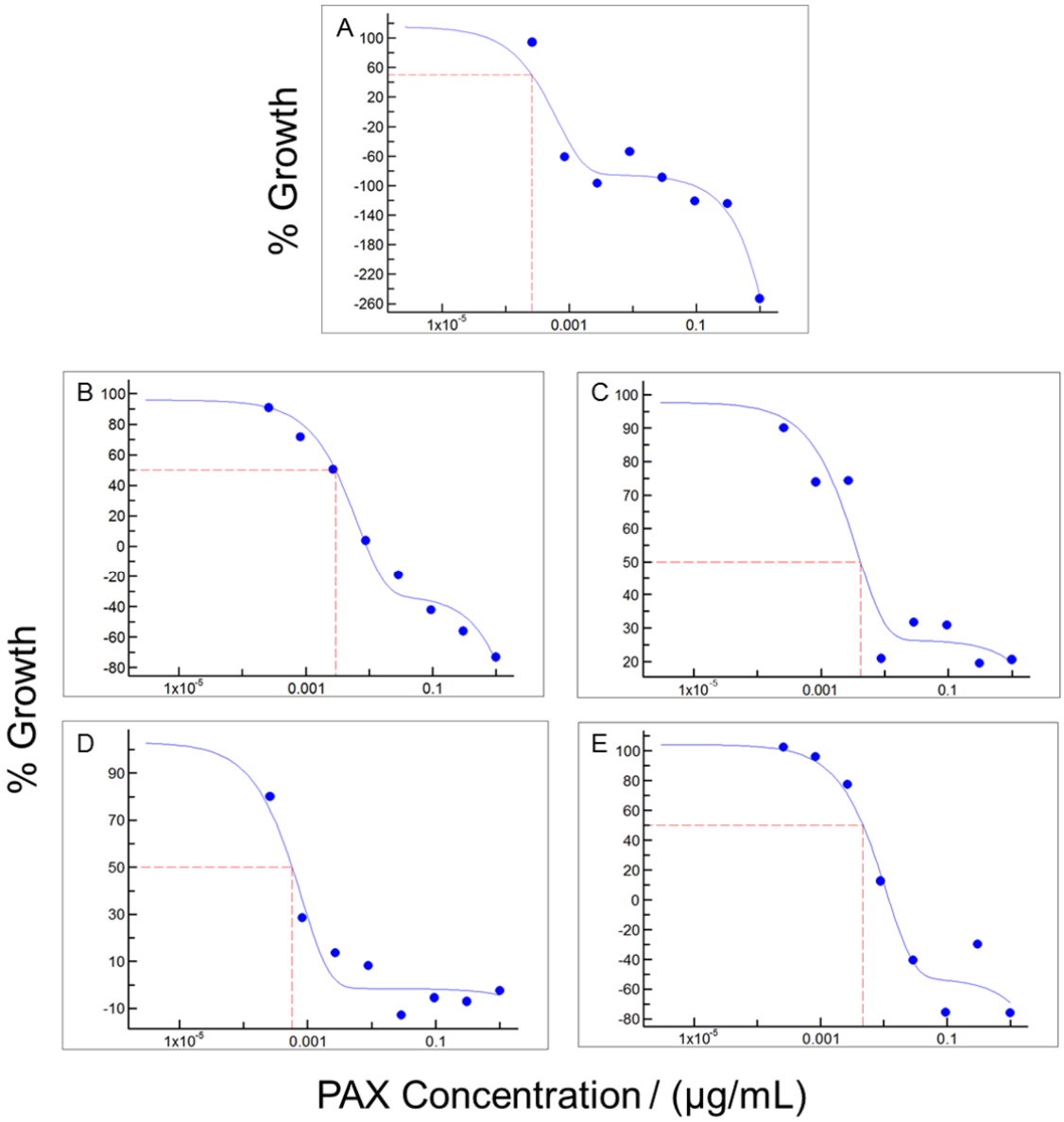

Figure S3. 72 h- Growth inhibition plots for (A) free PAX, (B) bulk-prepared PAX-loaded PNPs, and PAX-loaded PNPs prepared in the microfluidic reactor at different flow rates: (C) Q = 100 µL / min, (D) Q = 200 µL / min, and (E) Q = 400 µL / min. Data points in B-E represent mean data from triplicate PNP preparations under each condition. Dashed red lines indicate GI_{50} values.

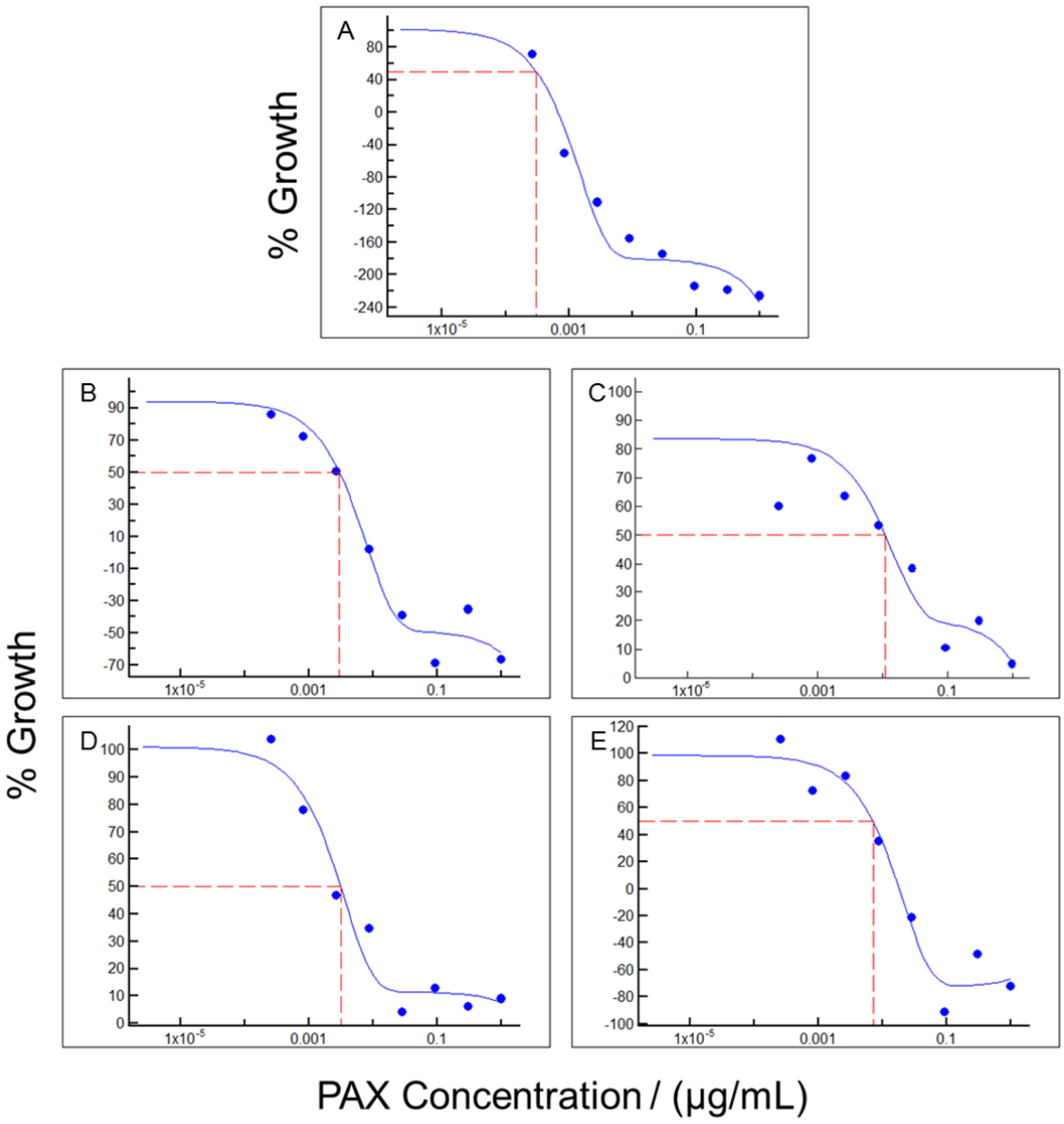

Figure S4. 96 h- Growth inhibition plots for (A) free PAX, (B) bulk-prepared PAX-loaded PNPs, and PAX-loaded PNPs prepared in the microfluidic reactor at different flow rates: (C) Q = 100 µL / min, (D) Q = 200 µL / min, and (E) Q = 400 µL / min. Data points in B-E represent mean data from triplicate PNP preparations under each condition. Dashed red lines indicate GI_{50} values.

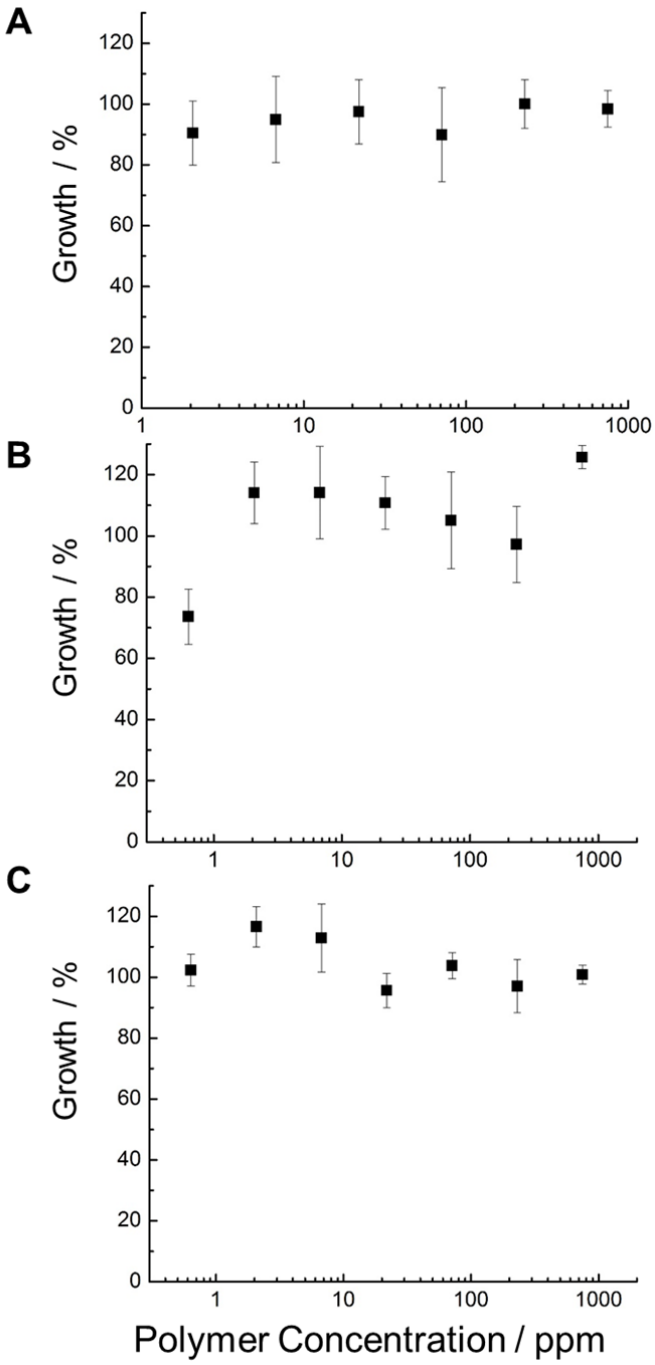

Figure S5. Percentage Growth plots for empty PCL2.1k PNPs from (A) 48 hours incubation, (B) 72 hours incubation, and (C) 96 hours incubation. Error bars were determined from triplicate preparations of PCL2.1k PNPs.

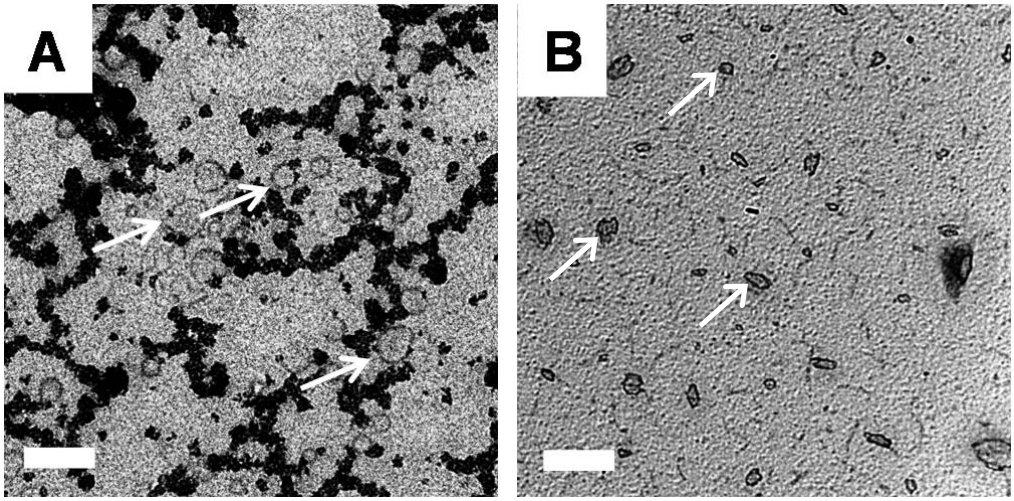

Figure S6. Representative unstained TEM images of vesicles present in the PCL2.1k Q = 200 μL/min condition (A), and the PCL12k Q = 200 μL/min condition (B). Scale bars are 100 nm.