Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B
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Supporting Information for

Paclitaxel-loaded polyphosphate nanoparticles: a potential strategy for bone cancer treatment

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Table S1. Mean diameter and zeta potential for the PPE nanoparticles with variable number of carbons between the phosphate groups, with and without staining with the fluorescent dye Bodipy.

Sample	Mean diameter ± S.D. (nm)	Zeta potential (mV)
PPE-NP-C6	352 ± 82	-64,3±6,3
PPE-NP-C10	88 ± 27	-59,2±11,8
PPE-NP-C20	138 ± 39	-47,4±12,5
BODIPY-PPE-NP-C6	286 ± 85	-62,3±6,8
BODIPY-PPE-NP-C10	84 ± 25	-67,4±13,9
BODIPY-PPE-NP-C20	143 ± 32	-47,7±12,4

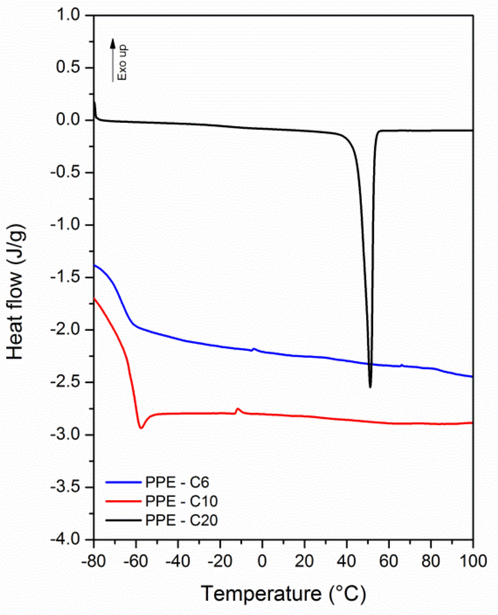

Fig. S1 DSC curves for of the saturated polyphosphoesters PPE-C6, PPE-C10 and PPE-C20.

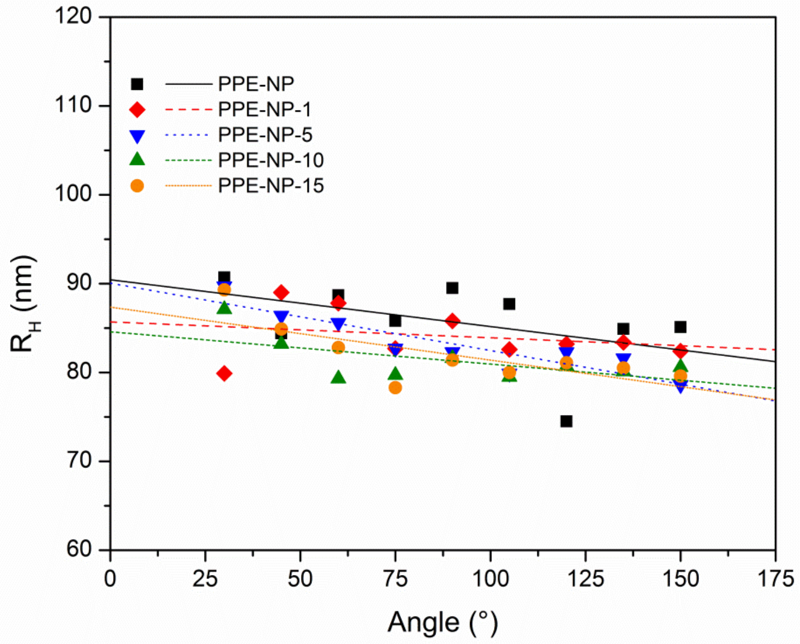

Fig. S2 Multi-angle DLS scattering of the "placebo" PPE nanoparticles and the Paclitaxel loaded PPE nanoparticles formulations.

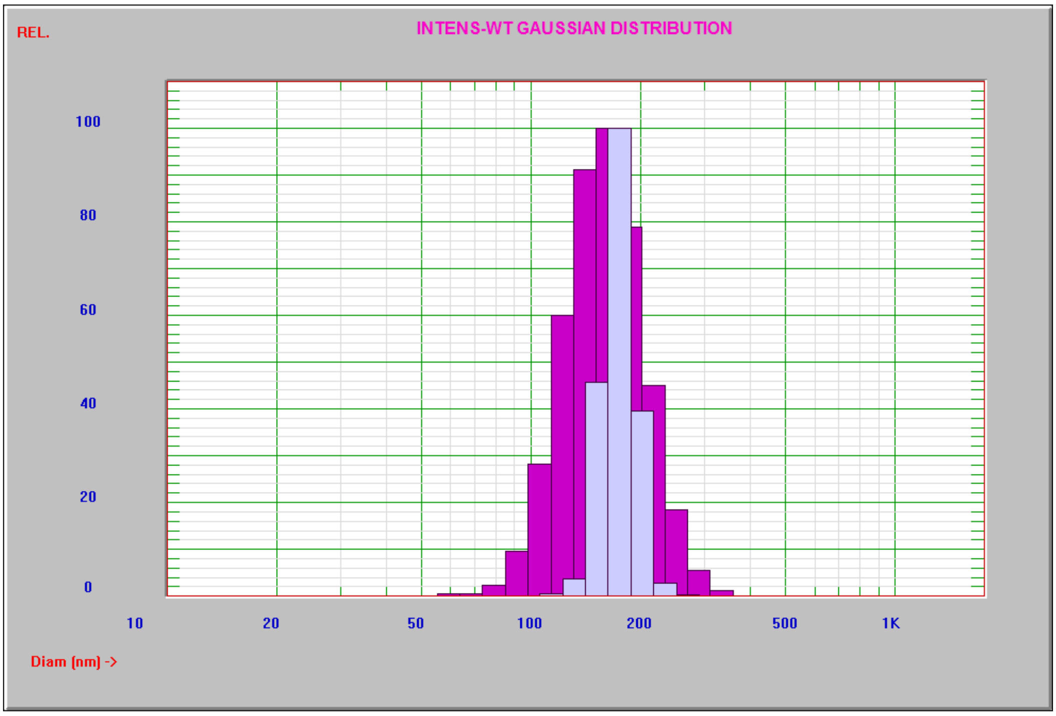

Fig. S3 DLS measurements of a first batch PPE-NP nanoparticles: before (blue) and after (pink) 2 months kept at 4ºC and pH 7.

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GPC-Results

Alexandrino
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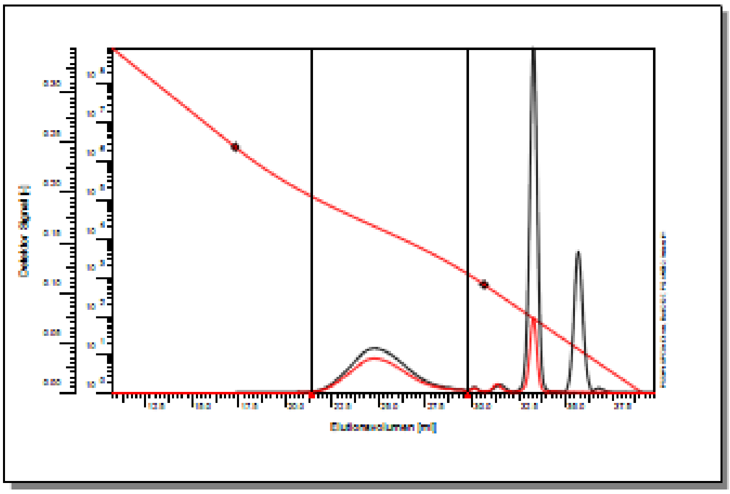
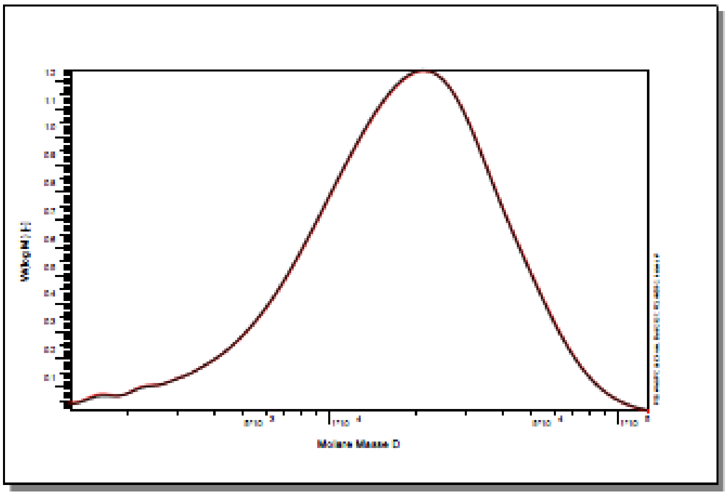
Elugram:



Calibration:	psthf05.CAL
Eluent:	THF
Flow rate:	1,0 ml/min
Pump:	PSS SECcurity
Inj.vol.:	100,00
Column1:	SDV 10e6
Column2:	SDV 10e4
Column3:	SDV 500
Temp.:	30,00 °C
Detector:	PSS SECcurity VWD
Detector:	Shodex RI 101

Molecular weight distribution:



PSS SECcurity VWD
Shodex RI 101

Name	Mn	Mw	D	Vp	Mp	Area
PSS SECcurit	11931,90	22688,60	1,90	24,77	21273,00	0,13
Shodex RI 10	11963,50	22624,40	1,89	24,79	21059,90	0,16

Fig. S4 GPC measurement for the polymer PPE-C20.

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GPC-Results

Alexandrino
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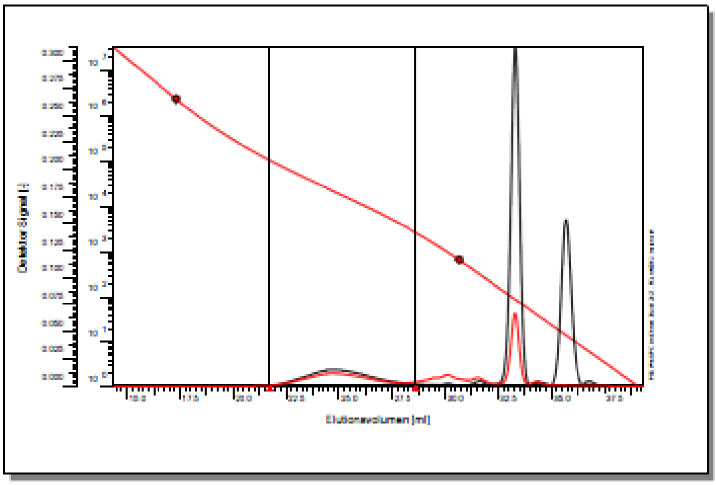
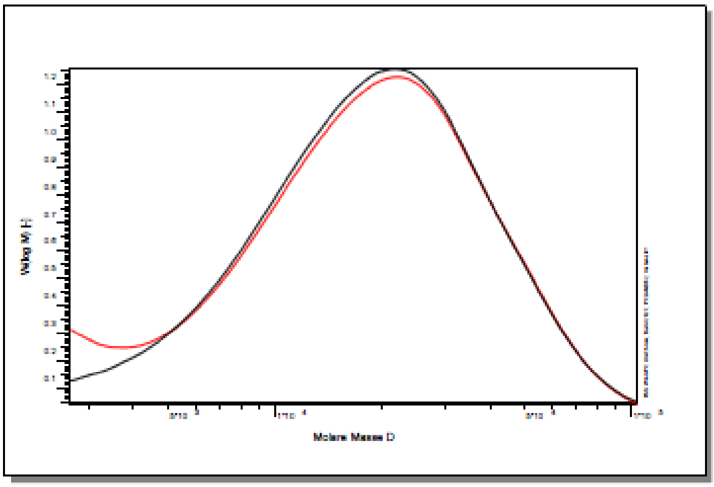
Elugram:



Calibration: psthf05.CAL
Eluent: THF
Flow rate: 1,0 ml/min
Pump: PSS SECcurity
Inj.vol.: 100,00
Column1: SDV 10e6
Column2: SDV 10e4
Column3: SDV 500
Temp.: 30,00 °C
Detector: PSS SECcurity VWD
Detector: Shodex RI 101

Molecular weight distribution:



PSS SECcurity VWD
Shodex RI 101

Name	Mn	Mw	D	Vp	Mp	Area
PSS SECcurit	12903,20	22962,50	1,78	24,71	21890,40	0,04
Shodex RI 10	13752,00	23216,90	1,69	24,73	21671,10	0,05

Fig. S5 GPC measurement for nanoparticles PPE-NP-C20 after 2 months kept at 4ºC and pH 7.

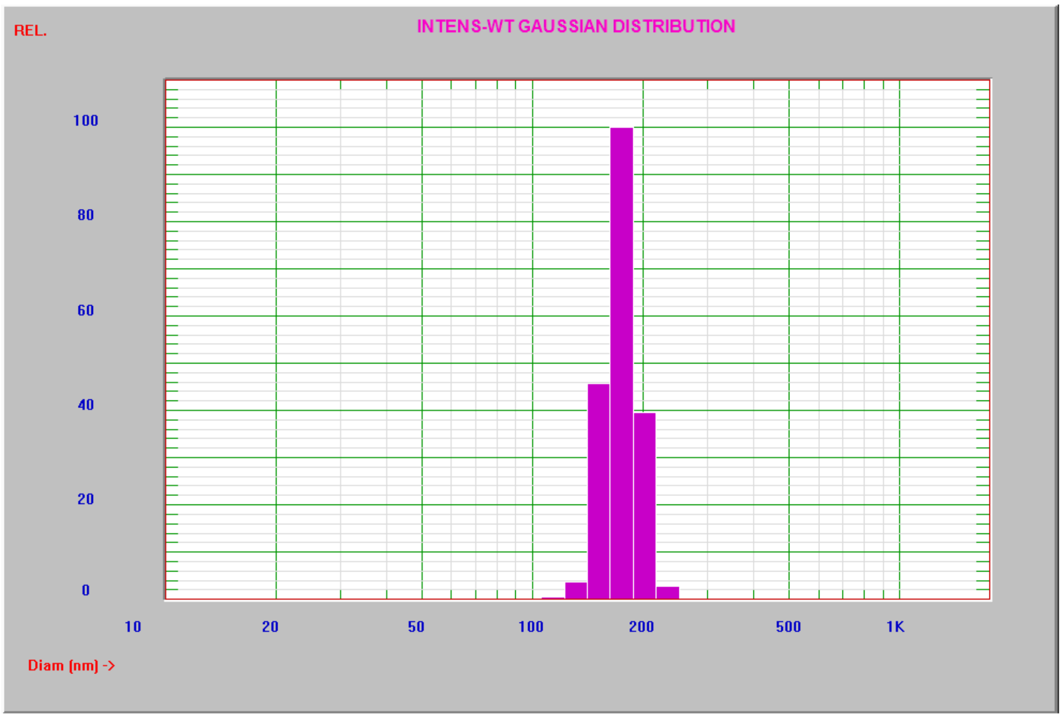


Fig. S6 DLS measurement: PPE-NP nanoparticles.

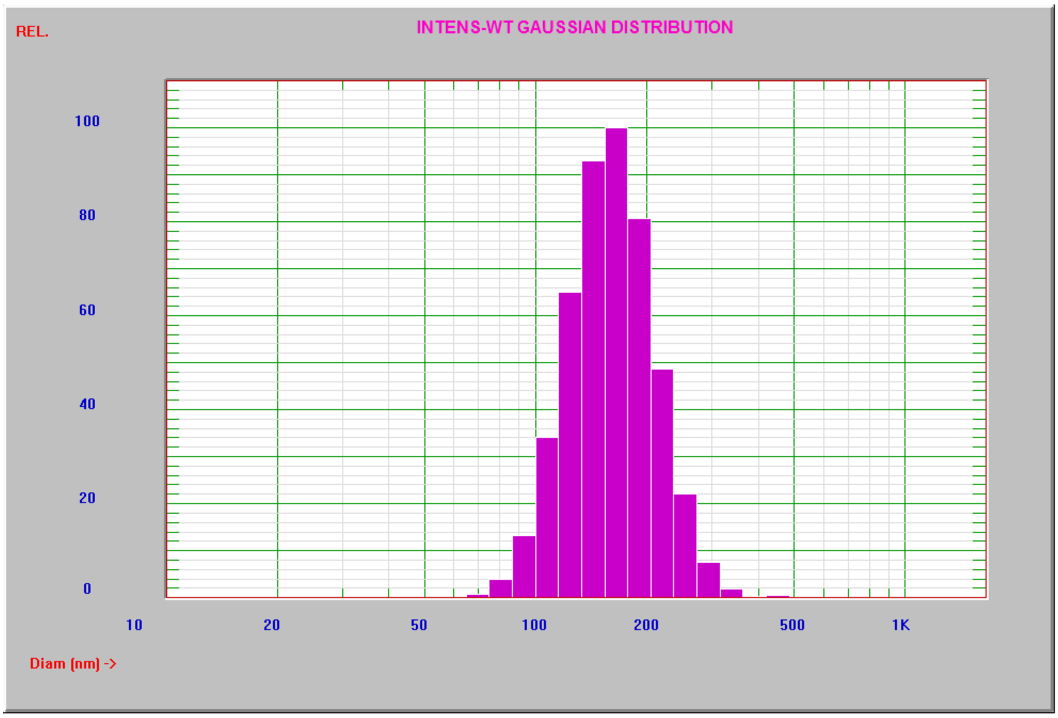


Fig. S7 DLS measurement: PPE-NP-1 nanoparticles.

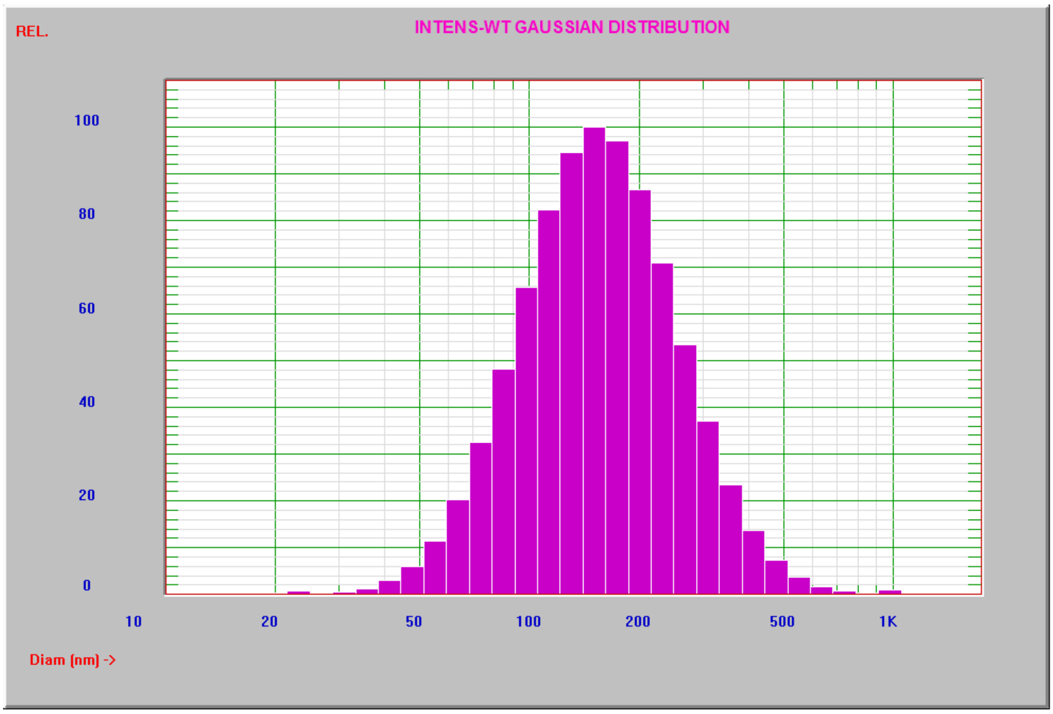


Fig. S8 DLS measurement: PPE-NP-5 nanoparticles.

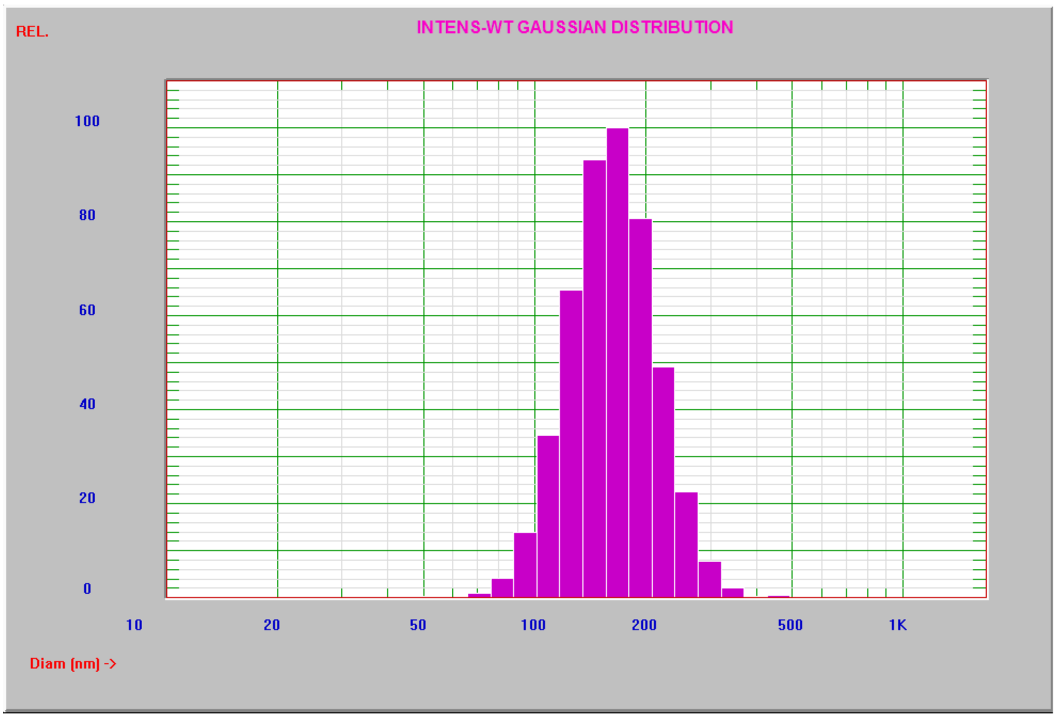


Fig. S9 DLS measurement: PPE-NP-10 nanoparticles.

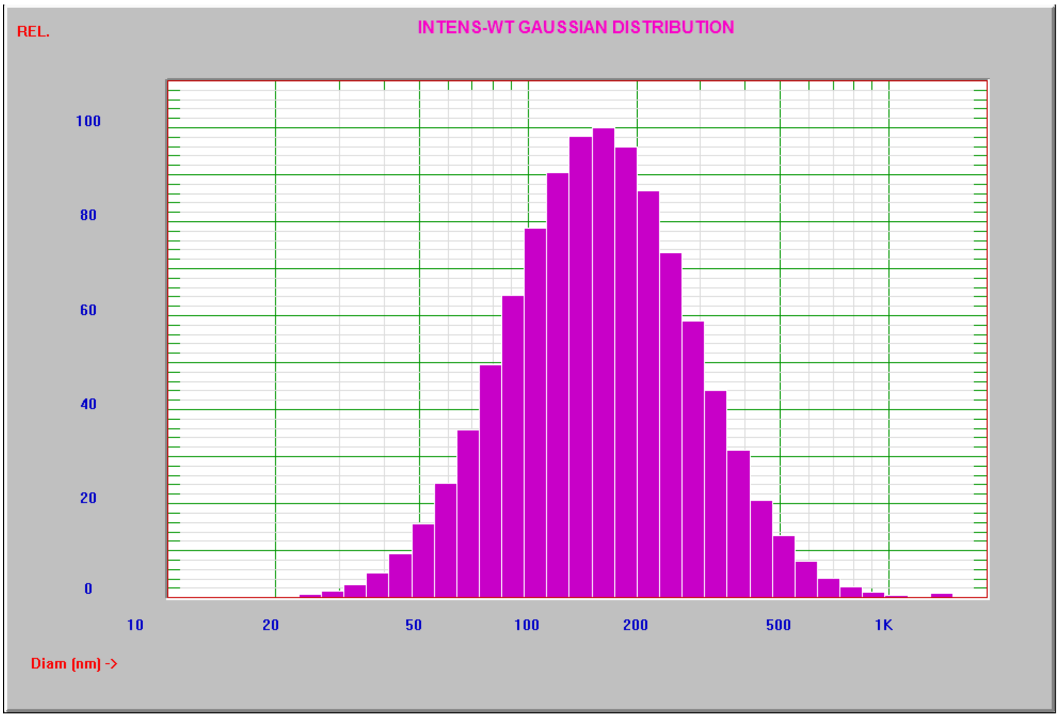


Fig. S10 DLS measurement: PPE-NP-15 nanoparticles.

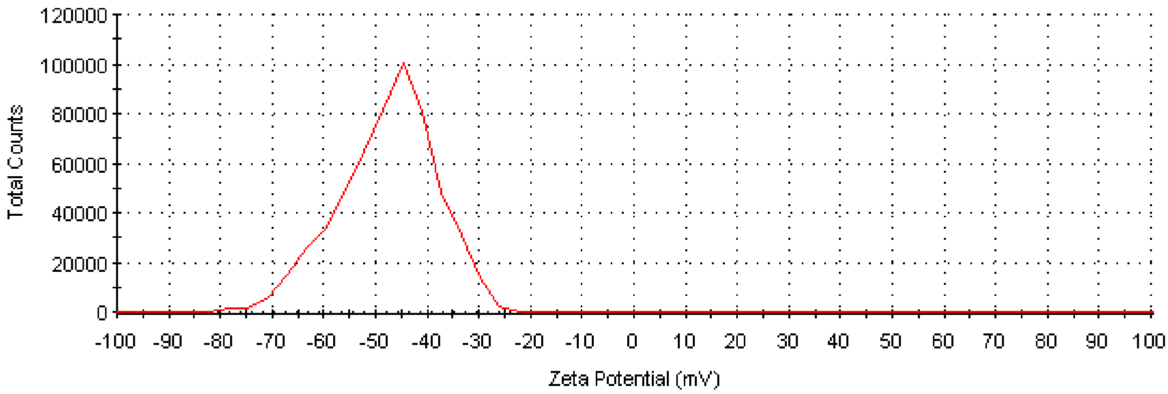


Fig. S11 Zeta-potential analysis from sample PPE-NP after dialysis.

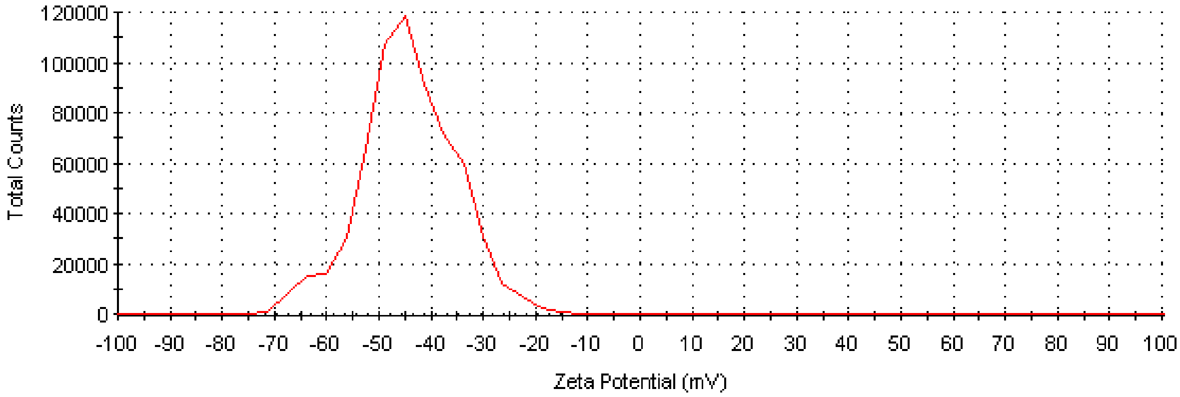


Fig. S12 Zeta-potential analysis from sample PPE-NP-1 after dialysis.

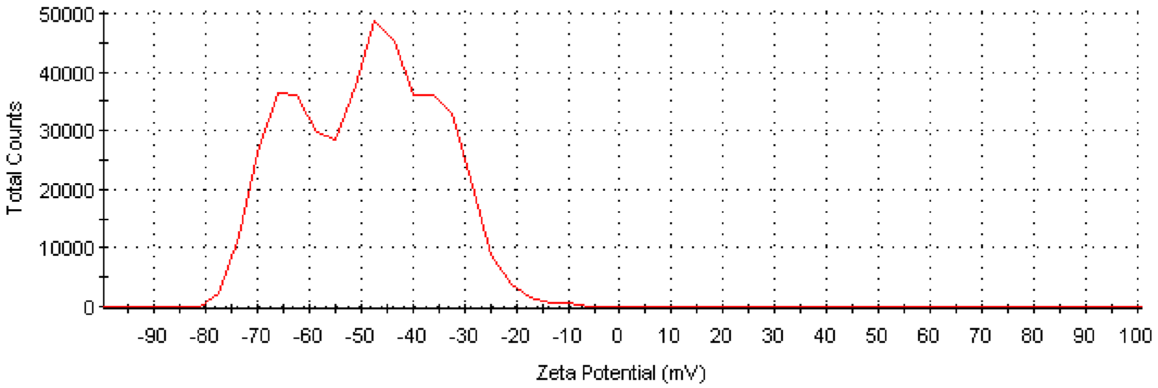


Fig. S13 Zeta-potential analysis from sample PPE-NP-5 after dialysis.

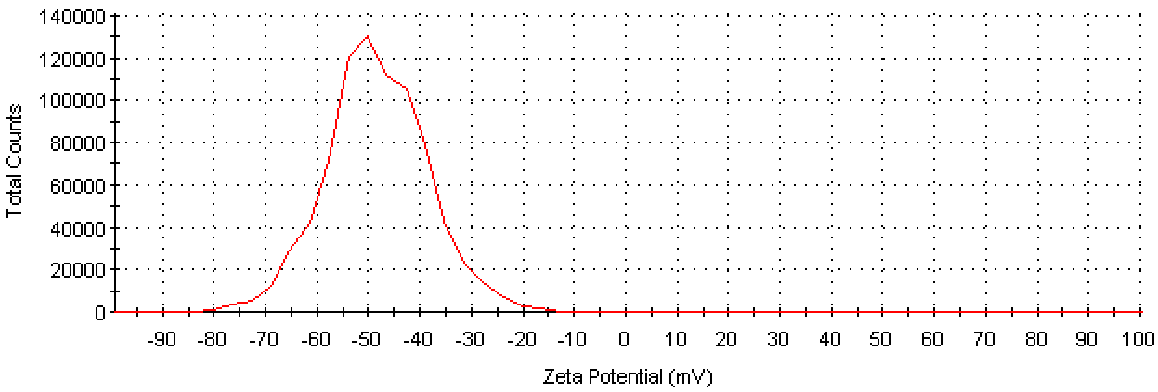


Fig. S14 Zeta-potential analysis from sample PPE-NP-10 after dialysis.

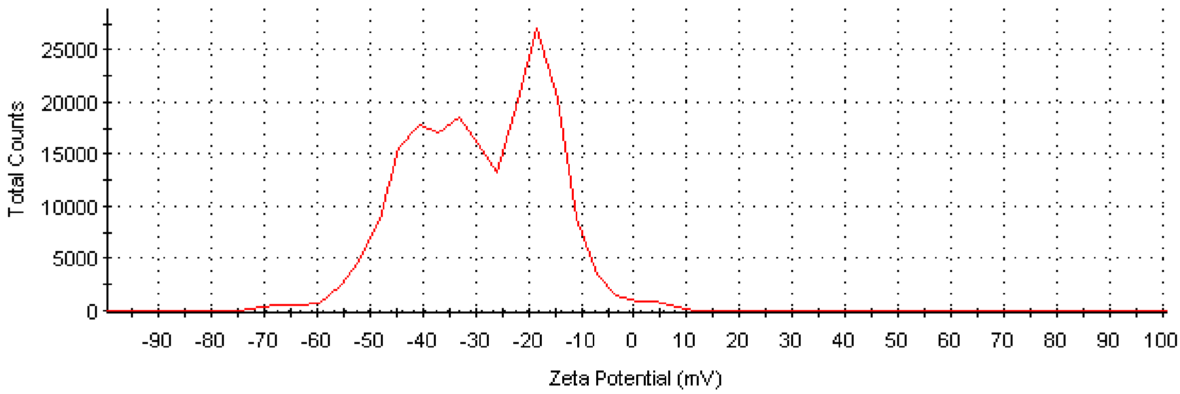


Fig. S15 Zeta-potential analysis from sample PPE-NP-15 after dialysis.

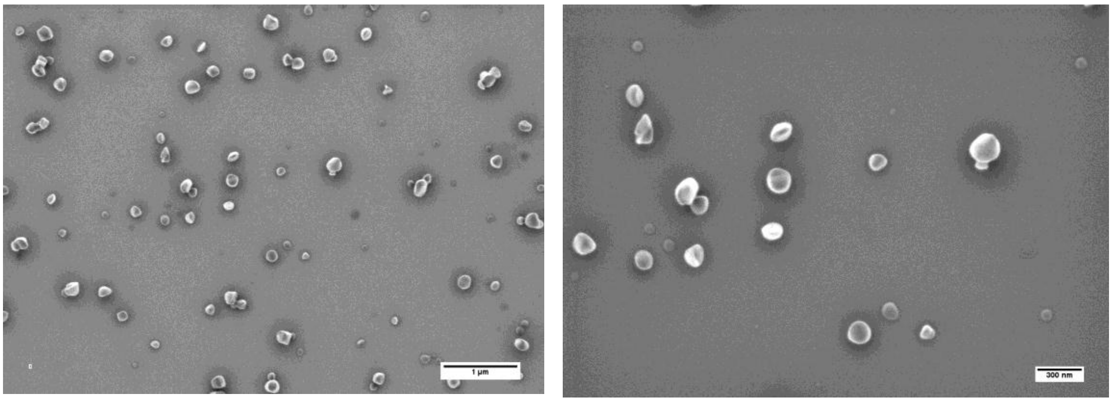


Fig. S16 SEM micrographies obtained from PPE-NP-5.

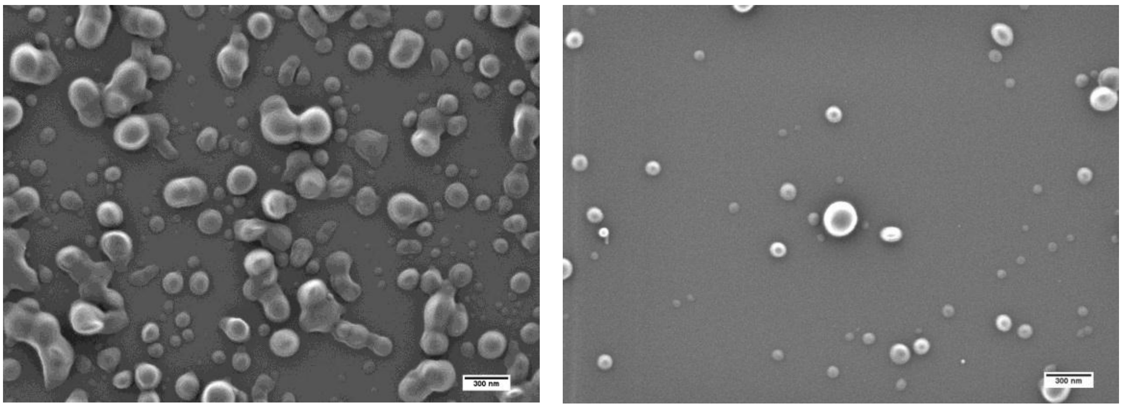


Fig. S17 SEM micrographies obtained from PPE-NP-15.

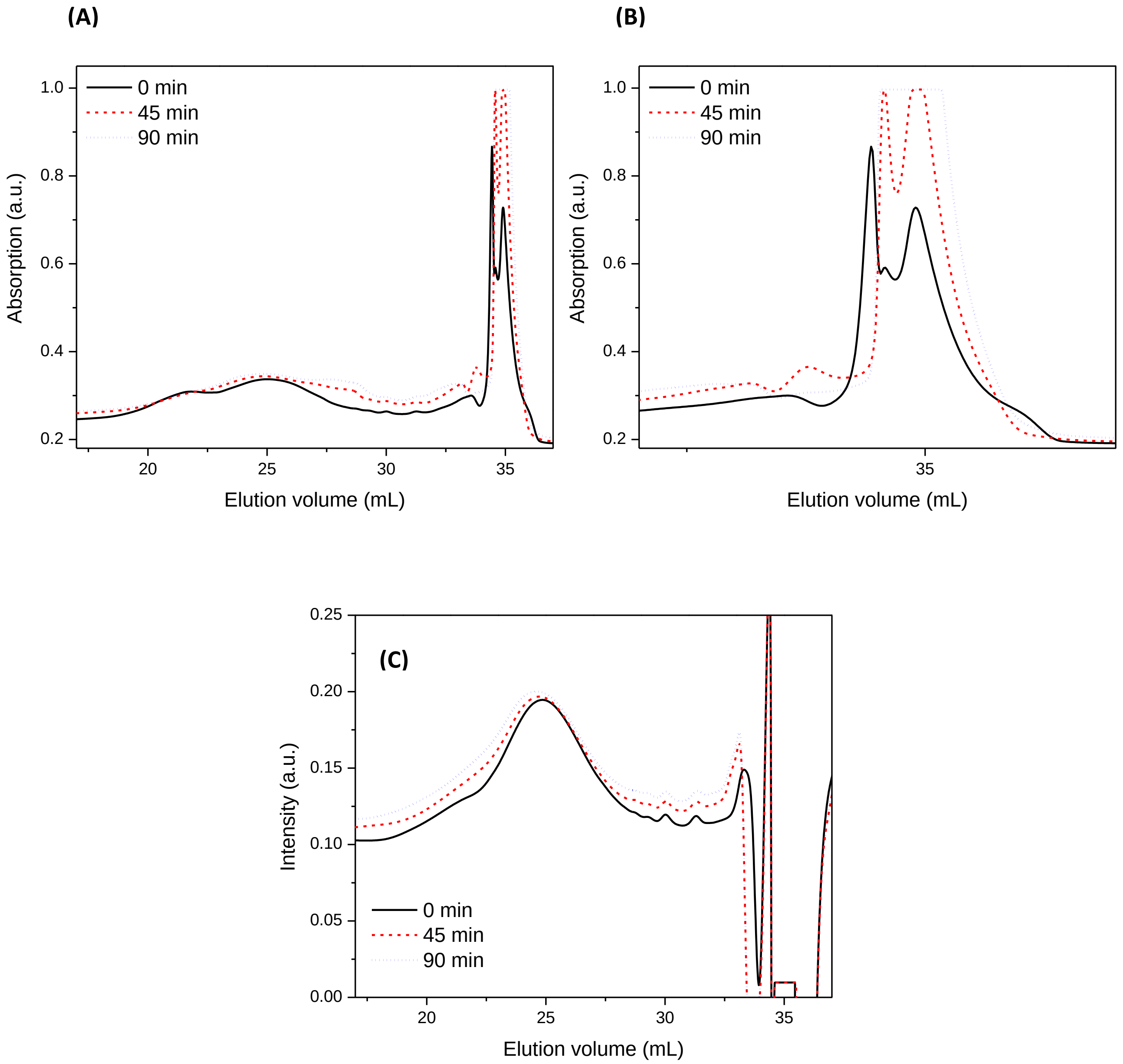


Fig. S18 GPC elugrams for the polymer PPE in THF and 0,1 M HCl: (a) and (b) UV-Vis signal; (c) Refractive Index signal.

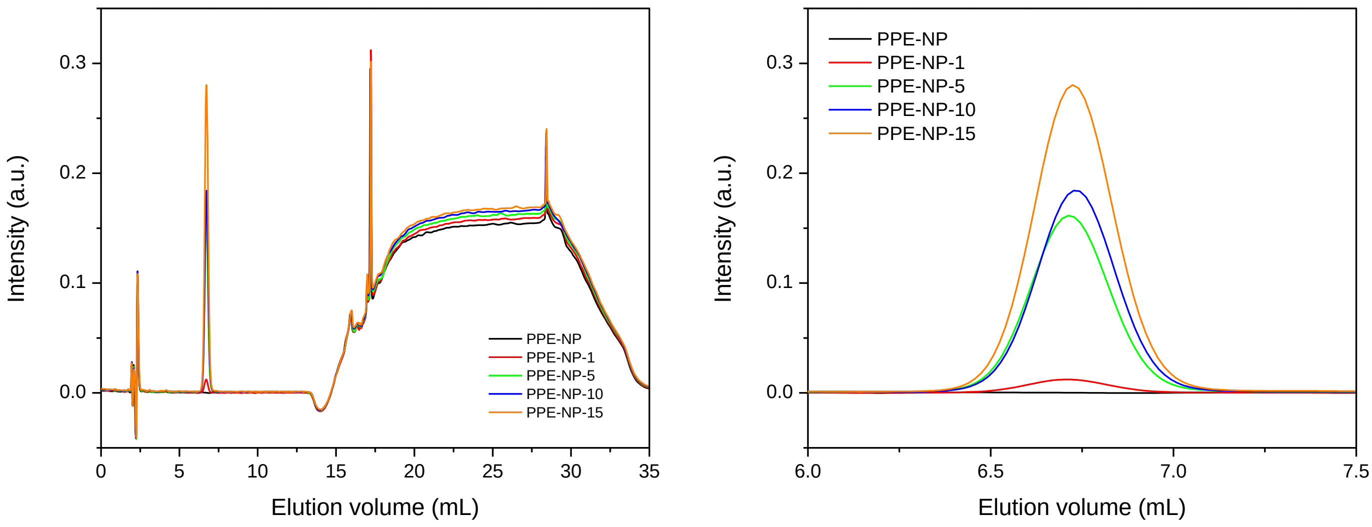


Fig. S19 HPLC calibration curve constructed from Paclitaxel in THF:0,1 % aqueous TFA (80:20) stock solutions.

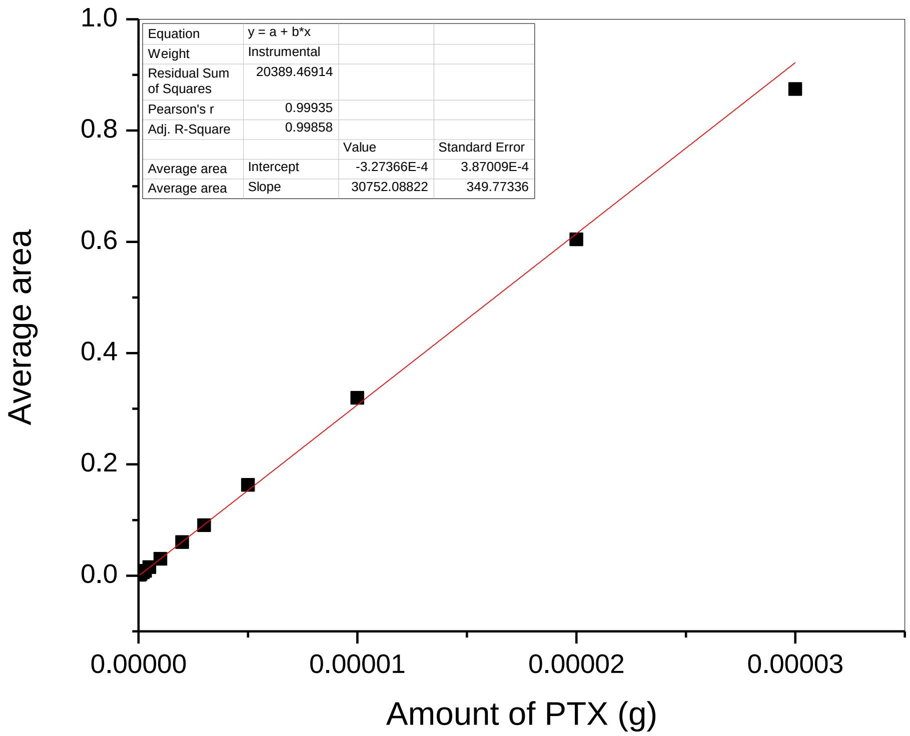


Fig. S20 HPLC calibration curve constructed from Paclitaxel in THF:0,1 % aqueous TFA (80:20) stock solutions.

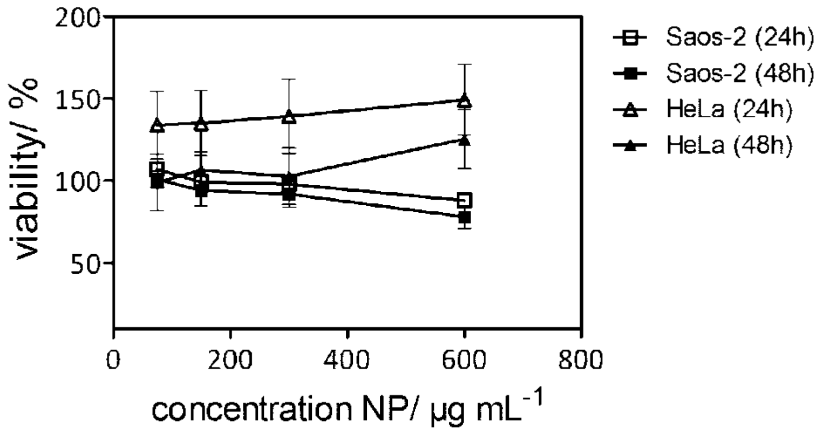


Fig. S21 Effect of polyphosphoester nanoparticles PPE-NPs on in vitro cell viability. (A) HeLa and Saos-2 cells treated with increasing concentrations of PPE-NP (75-600 µg/mL) for 24 h and 48 h. Cell viability was measured by CellTiter-Glo ® luminescence assay. Mean values ± standard deviation calculated from 4 replicates.

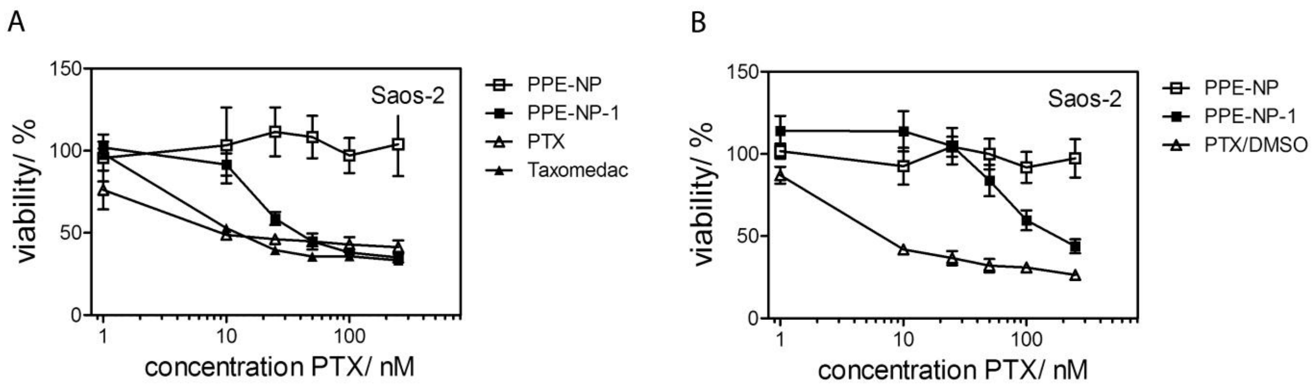


Fig. S22 Stability test of Paclitaxel loaded PPE-NP-1. The comparison of the dose response curves of freshly prepared PPE-NP-1 (A) and PPE-NP-1 after 3 month storage at 4°C in solution (B) revealed a slight decrease in efficiency. Cell viability of osteoblastoma cells tested with PrestoBlue in relation to untreated cells (100%). Mean values ± standard deviation calculated from 4 replicates.

Table S2. Surface attachment of different composed nanoparticles on biphasic calcium phosphate granules (MBCP+, 80-200 µm).

Nanoparticle	Composition	Functionalization	Surfactant	CaP attachment	Ref.
PPE-NP-C20	PPE-C20	-	-	yes	
PPE-NP-C10	PPE-C10	-	-	no	
PPE-NP-C6	PPE-C6	-	-	no	
PS-4	PS/C12-PET	phosphonate surfmer		yes	1
PLLA-Dop (CTMA-Cl)	PLLA	dopamin	CTMA-Cl	few	
PLLA-Dop (SDS)	PLLA	dopamin	SDS	no	
PLLA-Dop (HES)	PLLA	dopamin	HES200	no	
PBCA (CTMA-Cl)	PBCA	-	CTMA-Cl (0.1%)	no	
PBCA (SDS)	PBCA	-	SDS (0.1%)	no	
PBCA (AT50)	PBCA	-	AT50 (0.1%	no	

polyphosphoester (PPE), polystyrene (PS), 12-Methacrylamidododecylphosphonic acid (C12-PET), poly(L-lactide) (PLLA), hydroxyethyl starch (HES200), poly*(*butyl cyanoacrylate*)* (PBCA), polyethylenglycol, Lutensol (AT50), sodium dodecyl sulfate (SDS), cetyltrimethylammonium chloride (CTMA-Cl), dopamine (Dop)

1. Sauer, R.; Froimowicz, P.; Scholler, K.; Cramer, J. M.; Ritz, S.; Mailander, V.; Landfester, K., Design, Synthesis, and Miniemulsion Polymerization of New Phosphonate Surfmers and Application Studies of the Resulting Nanoparticles as Model Systems for Biomimetic Mineralization and Cellular Uptake. *Chemistry-a European Journal* **2012,** *18* (17), 5201-5212.

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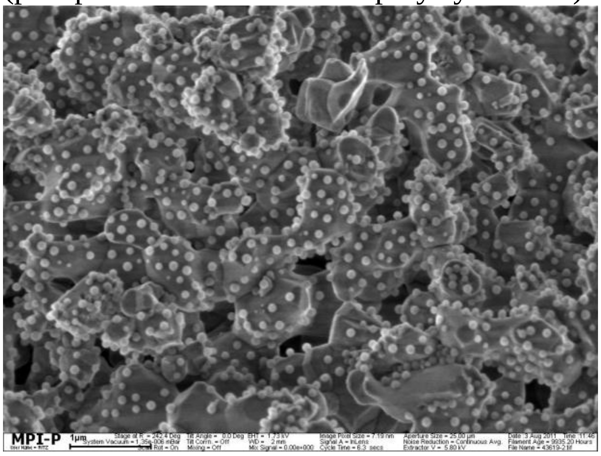
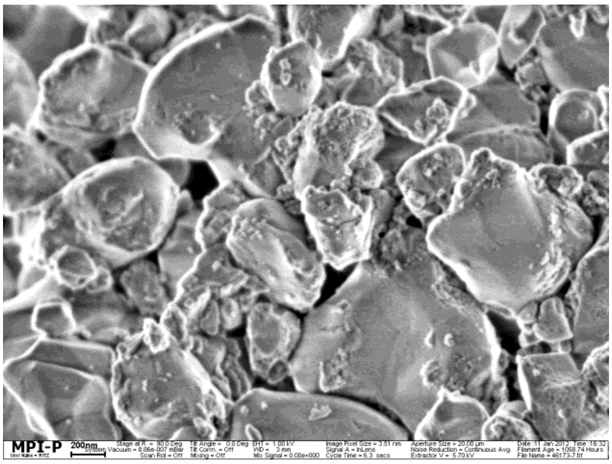
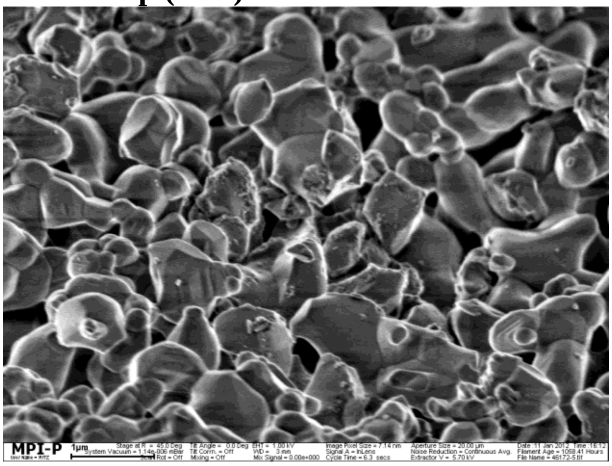
PS-4
(phosphonate functionalized polystyrene NP)



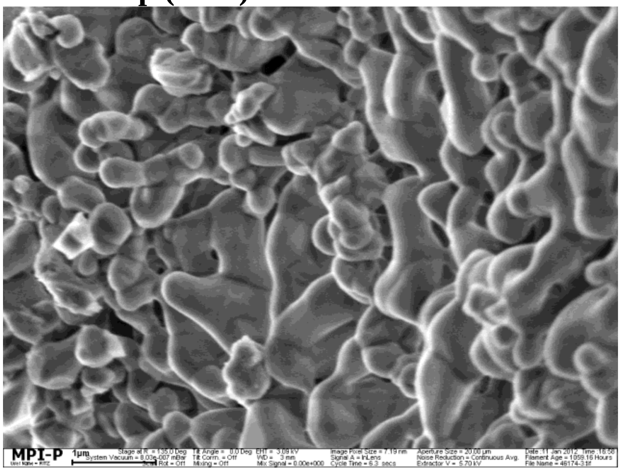
PLLA-Dop (CTMA-Cl)



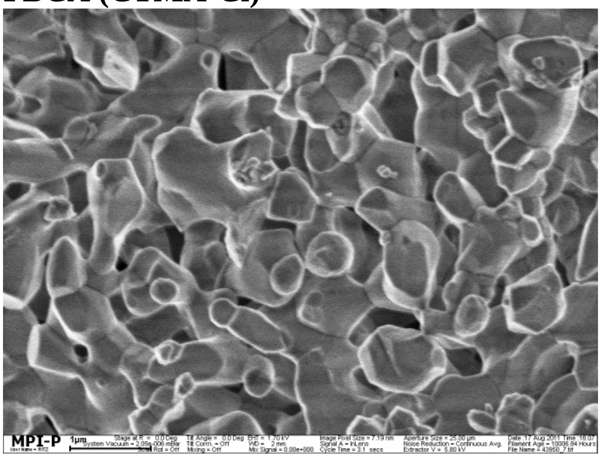
PLLA-Dop (SDS)



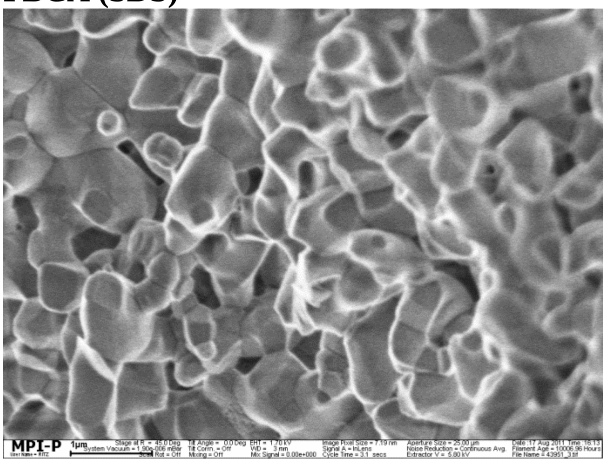
PLLA-Dop (HES)



PBCA (CTMA-Cl)



PBCA (SDS)



PBCA (AT-50)

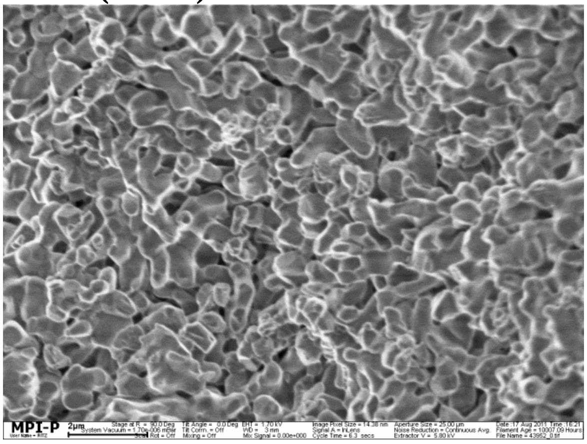


Fig. S23 SEM images of biphasic calcium phosphate granules, MBCP+ (80-200 µm) probed with different nanoparticles.

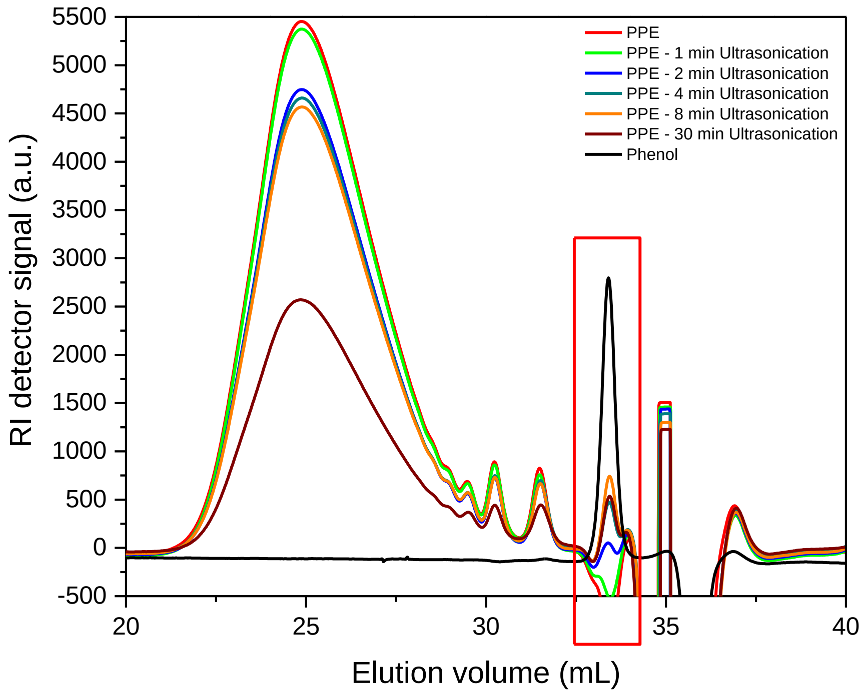


Fig. S24 RI detector signal from the GPC elugram for the polymer PPE after the application of ultrasound (1/4" tip; 70% amplitude; 30 s on and 10 s off) for different periods of time and for phenol.

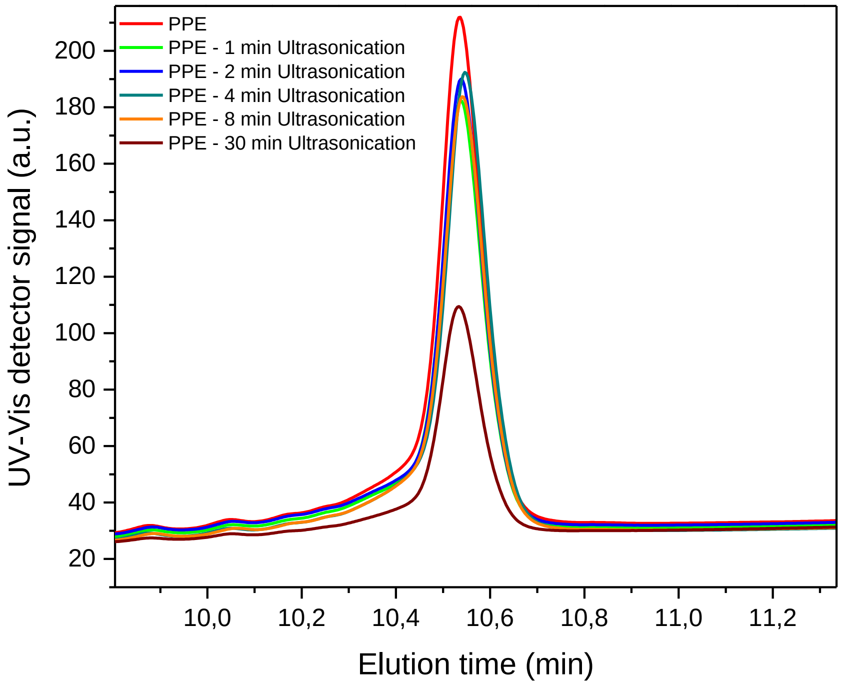


Fig. S25 UV-Vis detector signal from HPLC elugram for the polymer PPE after the application of ultrasound (1/4" tip; 70% amplitude; 30 s on and 10 s off) for different periods of time.

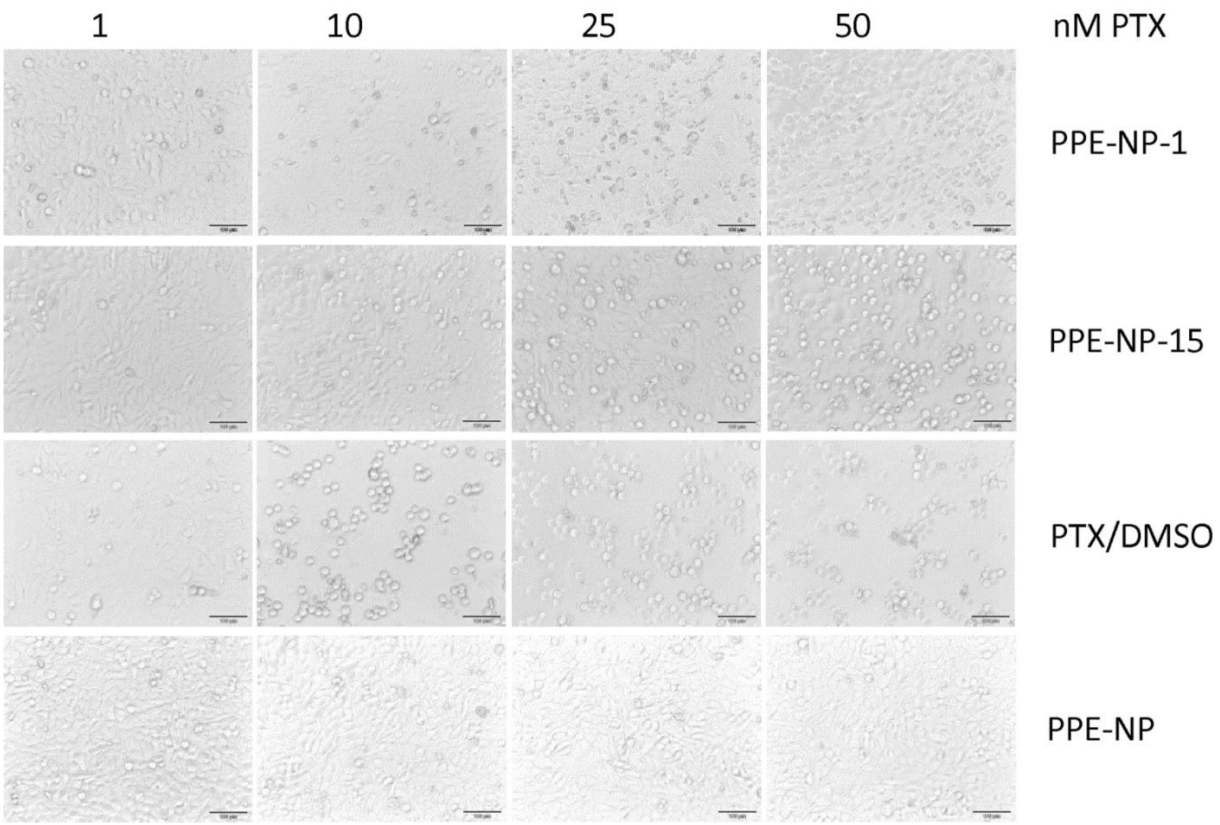


Fig. S26 Bright field images of HeLa cells treated with increasing concentrations of PTX and PTX loaded PPE-NPs (24h). Cells treated with the control nanoparticle PPE-NP showed a dense cell layer, whereas cells treated with PTX and PTX loaded nanoparticles showed a round, detached morphology, supporting the cell viablity studies with Prestoblue assay. Bar scales 100 µm.

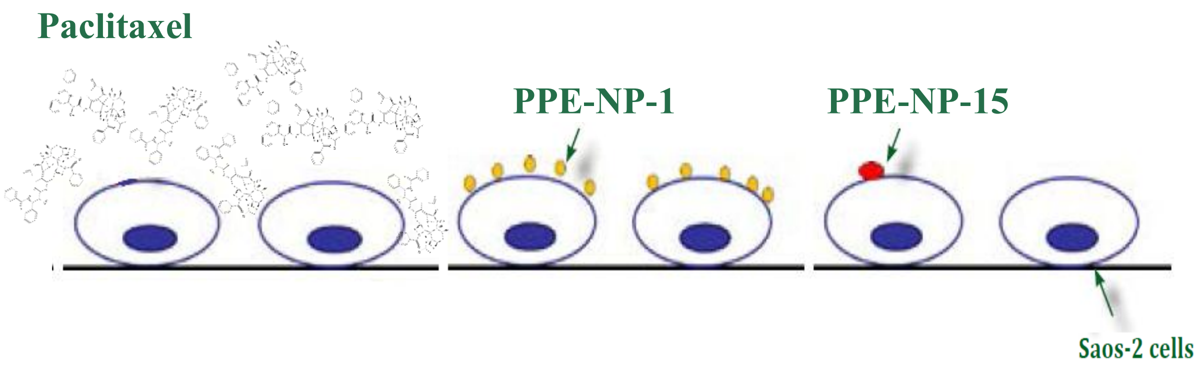


Fig. S27 Effectiveness of the nanoparticles systems: in an experiment controlled by the Paclitaxel concentration the number of nanoparticles of the low concentrated formulation (PPE-NP-1) is much bigger than of the high Paclitaxel concentrated particles (PPE-NP-15) for an experiment, what lowers the effectiveness of the higher concentrate system. The same reasoning can be applied for the comparison between nanoparticles systems and free Paclitaxel in solution.