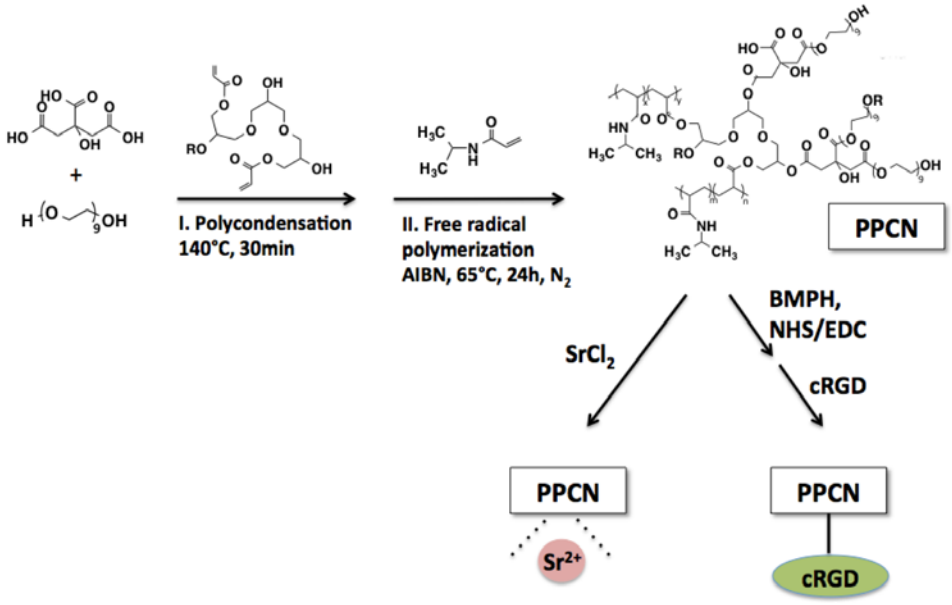
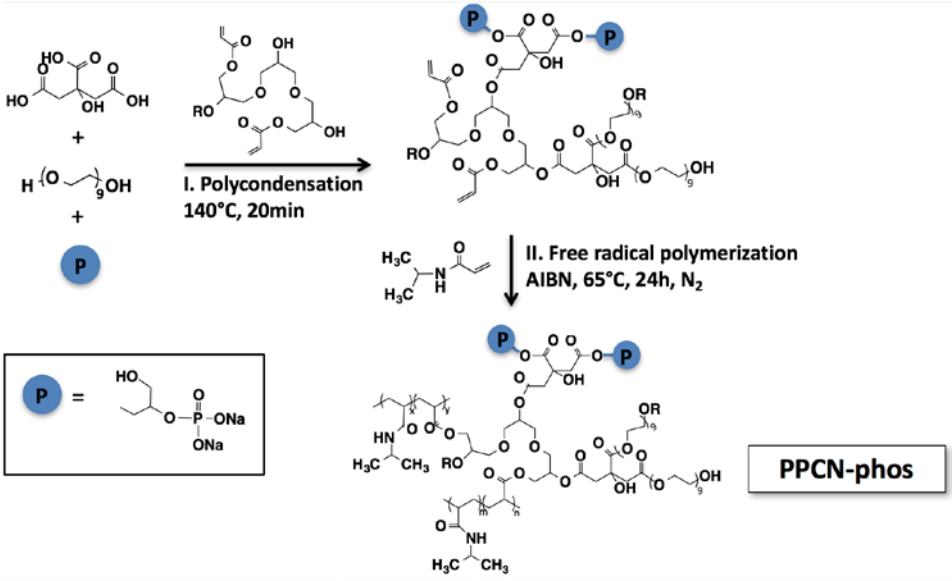
SUPPLEMENTARY FIGURES FOR JBMR PART A MANUSCRIPT



Scheme S1. Synthetic scheme of PPCN-Sr and PPCN-cRGD syntheses. PPCN was prepared by polycondensation and subsequent free radical polymerization as previously reported in Yang et al. Then, PPCN-cRGD gels, were prepared by covalently conjugating cyclic RGDfC peptide via maleimide chemistry to the available carboxylic acid groups of citric acid within the PPCN polymer chain. To prepare PPCN-Sr gels, PPCN was dissolved in PBS (1x) at 100 mg/ml. 100mM of $SrCl_2\,6H_2O$ was mixed into PPCN/PBS solutions and left to crosslink overnight at 4°C prior to use.



Scheme S2. Synthetic scheme of PPCN-phos synthesis. β-glycerophosphate was added during the polycondensation step of PPCN synthesis in 0.1 or 0.2 molar ratios. The proposed locations

of phosphate attachment are shown—specifically via reaction between the reactive hydroxyls of β-glycerophosphate and the available carboxyl groups of citric acid. PPCN-phos of 0.1 molar ratio was used in subsequent cell studies because higher molar ratios exhibited overcrosslinking.

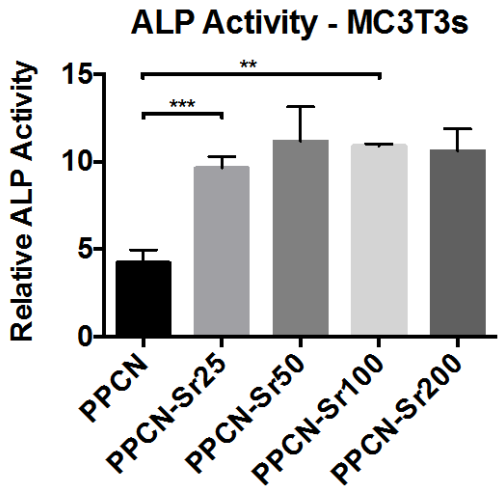


Figure S1. Day 4 alkaline phosphatase measurements of MC3T3 cells in 3D culture. Cells were seeded in PPCN-Sr gels of varying $SrCl_2$ concentrations from 25mM to 200mM to clearly show PPCN-Sr-induced ALP effect. ** and *** denote *p*-values <0.01 and <0.001, respectively.

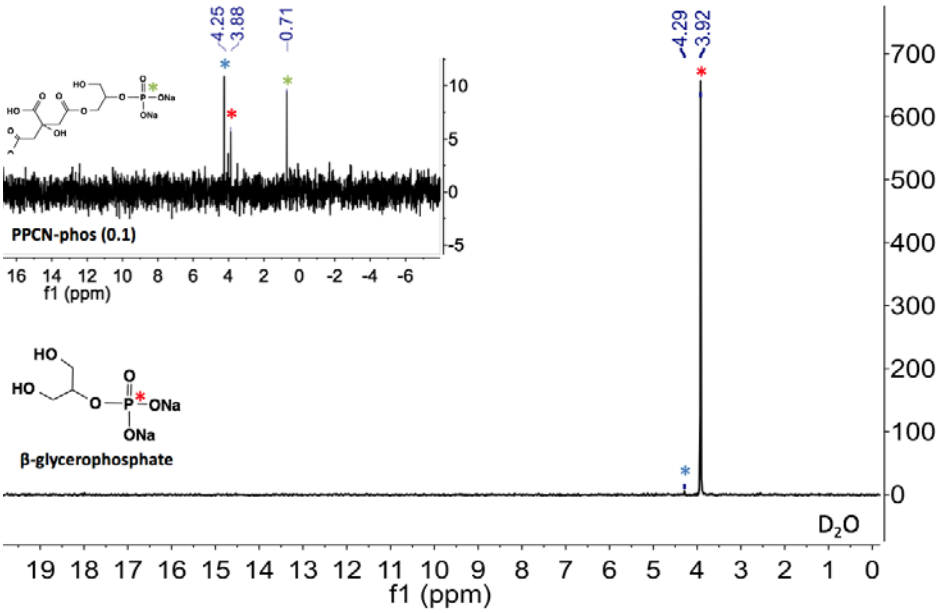


Figure S2. ^{31}P NMR of β-glycerophosphate alone and (inlay) PPCN-phos. Main peak associated with phosphate is seen at 3.92 ppm in former and 3.88 ppm in latter. The appearance of a new peak

at 0.71 ppm is attributed to the conjugation of β-glycerophosphate. The signal at 4.29 ppm which appears again at 4.25 ppm in the PPCN-phos spectrum is due to a slight impurity in the original reagent material, hypothesized to be a phosphate dimer. ^{31}P NMR spectrum of PPCN-phos was recorded using an automated 400 MHz Agilent DD MR-400 spectrometer at ambient temperature, using D_2O as a solvent.

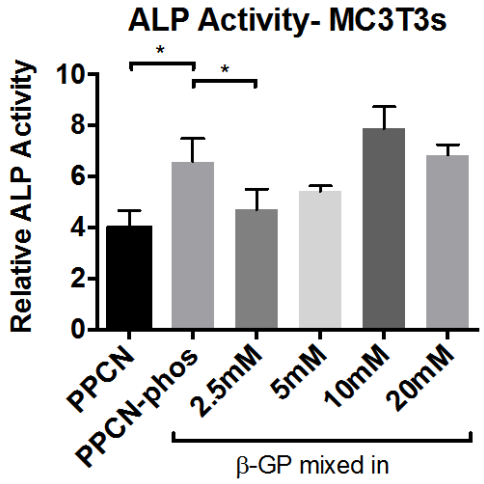


Figure S3. Day 4 alkaline phosphatase activity compares conjugated phos with mixed β-glycerophosphate at various concentrations. PPCN-phos contains approximately 2.5 mM final concentration of phosphate. Other materials were prepared with 2.5 mM, 5 mM, 10 mM and 20 mM phosphate mixed in following regular PPCN synthesis. MC3T3s were seeded into these various gels and tested for ALP at day 4. These results showed that cells seeded in PPCN-phos had significantly higher ALP activity than cells seeded in the comparable material where 2.5 mM was mixed in. This suggests that the covalent conjugation may allow phosphate to persist for longer in the material as compared to the mixed materials where regular media changes cause mixed phosphate to be removed.

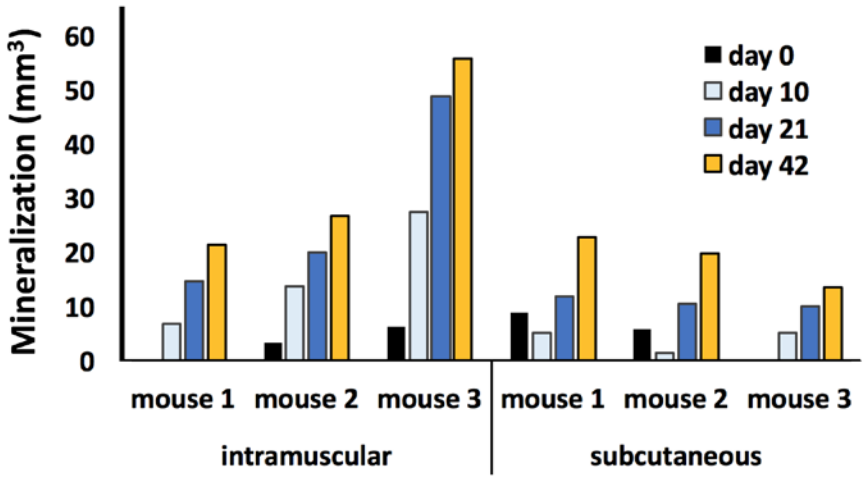


Figure S4. PPCN-Sr/hMSCs induced mineralization was quantified from the microCT data. Data is shown per each mouse for both subcutaneous and intramuscular groups. A 100 ul intramuscular injection resulted in higher mineralization levels as compared to the same volume injected via subcutaneous route. Day 0 represents the initial 100 ul of PPCN-Sr material detected via microCT. This value differs slightly among mice due to human injection error. However, it is consistently shown that a higher initially detectable injection volume leads to greater overall mineralization over a 6 week time course.

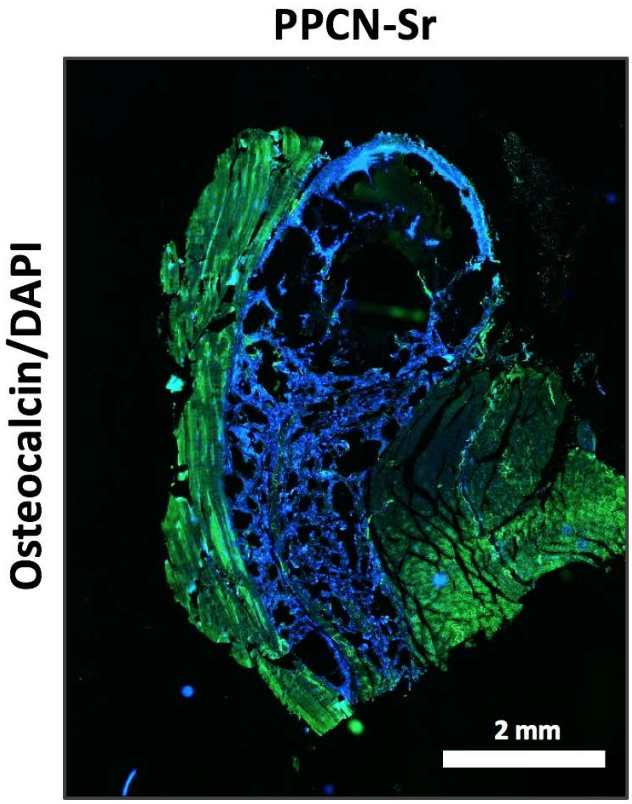
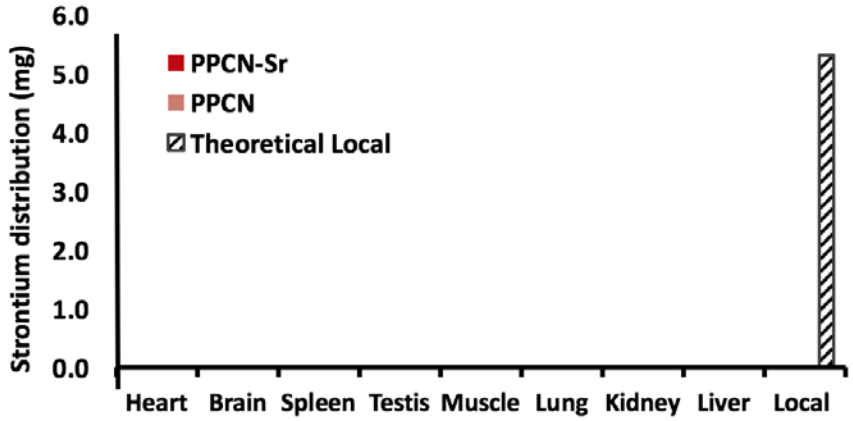


Figure S5. Full stitched tissue section of muscle injection site of PPCN-Sr shows enhanced osteocalcin staining throughout the section (green) and significant cell infiltration (DAPI, blue).

a.



b.

	PPCN	PPCN-Sr
	Atomic %	*Atomic %*
C	48.47	68.57
Ca	0.00	3.98
N	0.00	2.74
O	1.26	19.83
P	0.00	2.88
Si	0.29	2.00
Sr	0.00	0.00

XPS Elemental Analysis

Figure S6. Day 42 ICP analysis of 8 mouse organs shows that strontium is not present in any of the main organs upon digestion (a). Additionally, XPS analysis of sectioned muscle tissue show that strontium is cleared and no longer present at the site of injection (b). XPS analysis shows calcium, phosphate and oxygen content consistent with mineralization in the PPCN-Sr group, but no local strontium remaining at week 6.

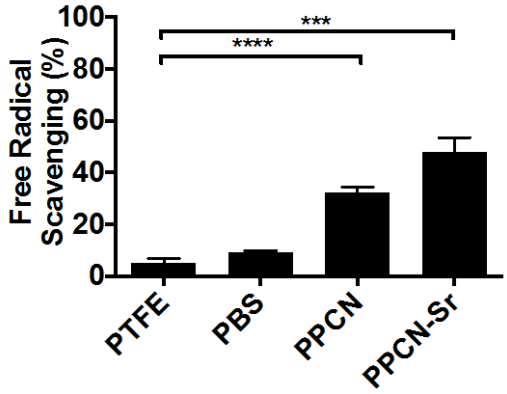


Figure S7. Sr^{2+} functionalization does not alter PPCN's antioxidant properties. 8 hour ABTS radical scavenging capacity for both PPCN and PPCN-Sr is significant as compared to PTFE.

SUPPLEMENTARY INFORMATION FOR JBMR PART A MANUSCRIPT

i. Viability and ALP Activity in Murine MC3T3 Osteoprogenitor Cells

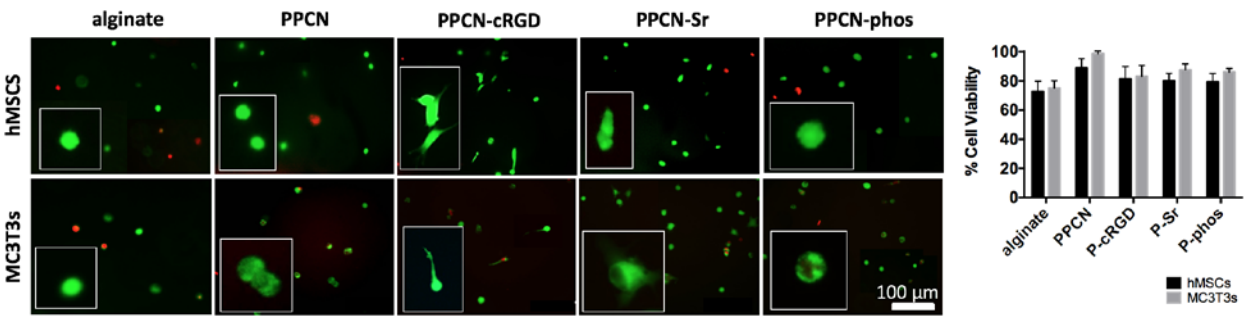


Figure S8. We conducted the viability studies in a second cell line to underscore the interspecies breadth of the macromolecule. Murine pre-osteoblast MC3T3-E1 cells (ATCC) were encapsulated in alginate, PPCN, PPCN-Sr, PPCN-phos or PPCN-cRGD solutions of 100 mg/mL PPCN in PBS (1x). Murine pre-osteoblast MC3T3-E1 cells (ATCC) were cultured in DMEM:F12, 1 g/L glucose and supplemented with 10% FBS and 5 ml 10x penicillin-streptomycin with no further osteogenic supplementation. MC3T3-E1 cells used were at passage 15 or below.

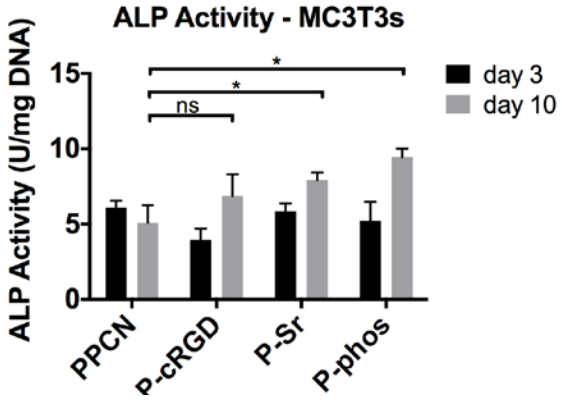


Figure S9. Alkaline Phosphatase activity was assayed as reported in the primary manuscript. MC3T3 cells entrapped in PPCN-Sr and PPCN-phos show enhanced ALP activity at day 10.

ii. Combining the three formulations cRGD, Sr, and phos does not produce greater osteoinduction

Our results also show that targeting multiple pathways at once does not necessarily result in enhanced osteodifferentiation. When we combined the three formulations into one gel, "PPCN-combo" by mixing 1:1 PPCN-phos and PPCN-cRGD and subsequently crosslinking 100 mM of $SrCl_2$ overnight, it did not result in higher ALP activity for the hMSCs. Additionally, it did not result in significantly higher OPN expression when tested at day 21. This data is not shown but we take it to suggest that simply combining three different routes of osteoinduction is not a substitute to employing each route individually.

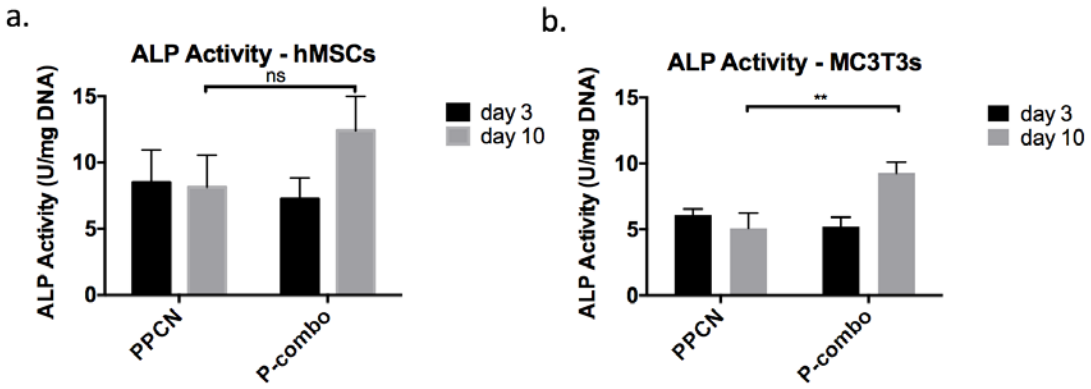


Figure S10. Day 3 and 10 alkaline phosphatase (ALP) activity compares PPCN with the combination gel (P-combo: P-cRGD-Sr-phos) for (a) hMSCs and (b) MC3T3s. No significant increase in ALP is detected for hMSCs at the timepoints tested. However, significant increase in ALP is detected for MC3T3s grown in combination gel as compared to control. ***P*-value<0.01.