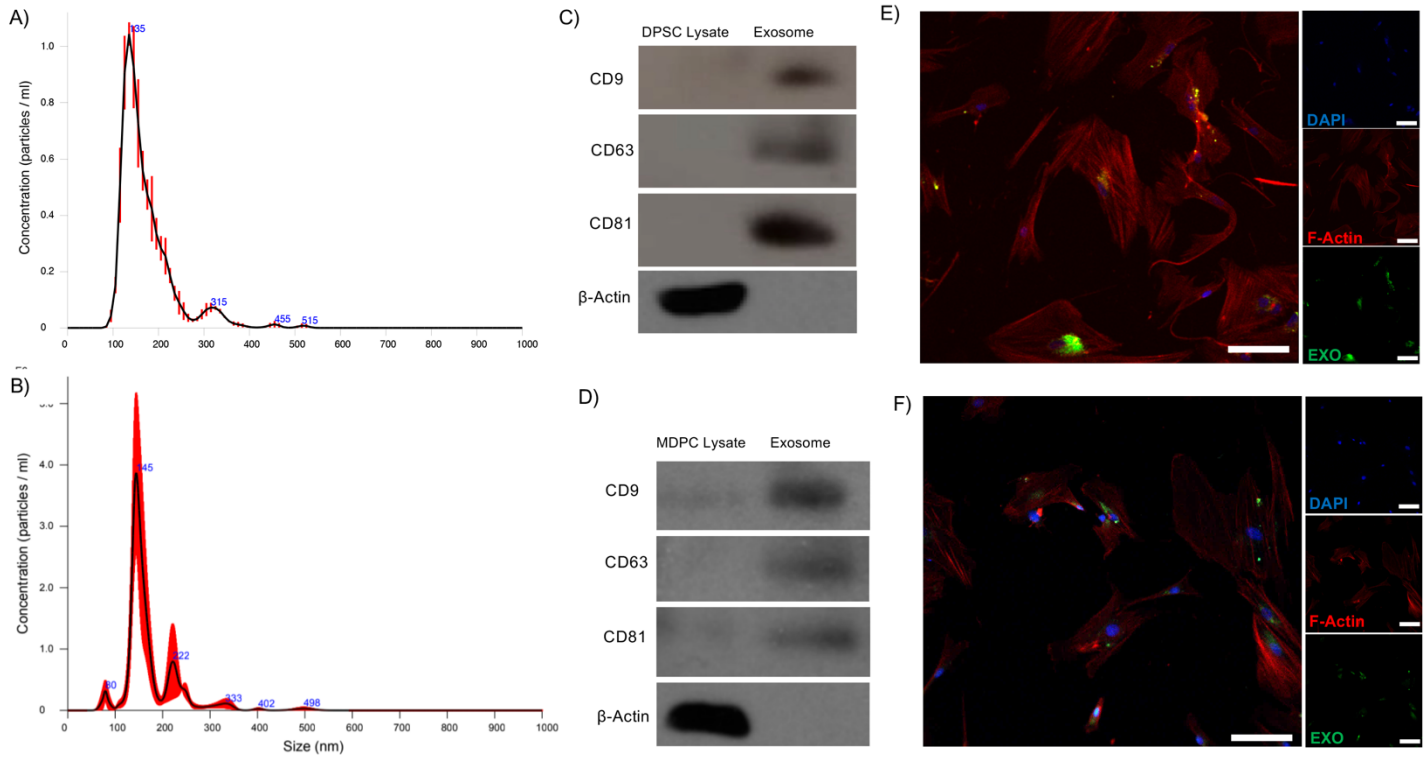
Supplementary Information

Controlled Release of Odontogenic Exosomes from a Biodegradable Vehicle Mediates Dentinogenesis as a Novel Biomimetic Pulp Capping Therapy

W. Benton Swanson, Ting Gong, Zhen Zhang, Miranda Eberle, David Niemann, Ruonan Dong, Kunal J. Rambhia, Peter X. Ma

Table 1. Primer sequences used for quantitative polymerase chain reaction evaluation of gene expression.

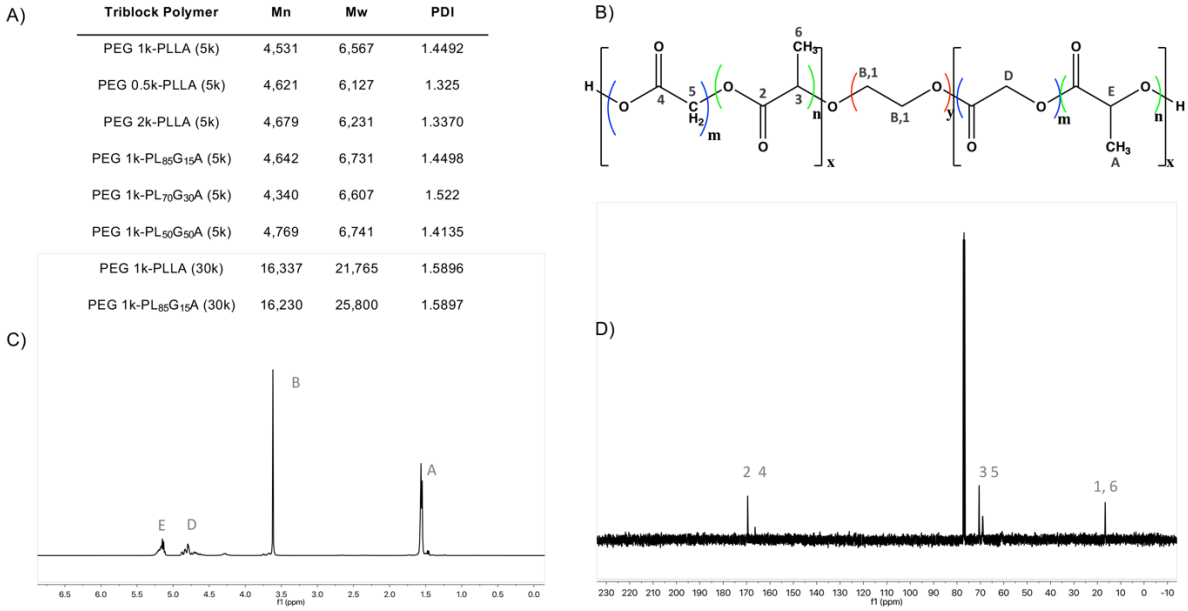
Gene	Forward Sequence	Reverse Sequence
Human GAPDH	CCATGGAGAAGGCTGGG	CAAAGTTGTCATGGATGACC
Human DSPP	GTGATAGAGGAAGGCAAGAG	ATTCCAGCCCTCAATATTCC
Human OCN	CAAAGGTGCAGCCTTTGTGTC	TCACAGTCCGGATTGAGCTCA
Human BSP	GCGAAGCAGAAGTGGATGAAA	TGCCTCTGTGCTGTTGGTACTG
Human VEGF	CAAAAACGAAAGCGCAAGAAA	GCGGGCACCAACGTACAC
Mouse GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Mouse Runx2	ATGCTTCATTCGCCTCACAAA	GCACTCACTGACTCGGTTGG
Mouse OCN	GAACAGACAAGTCCCACACAG	AGCAGAGTGAGCAGAAAGATG
Mouse DSPP	ATTCCGGTTCCCCAGTTAGTA	CTGTTGCTAGTGGTGCTGTT
Mouse Col1a1	GCTCCTCTTAGGGGCCACT	ATTGGGGACCCTTAGGCCAT



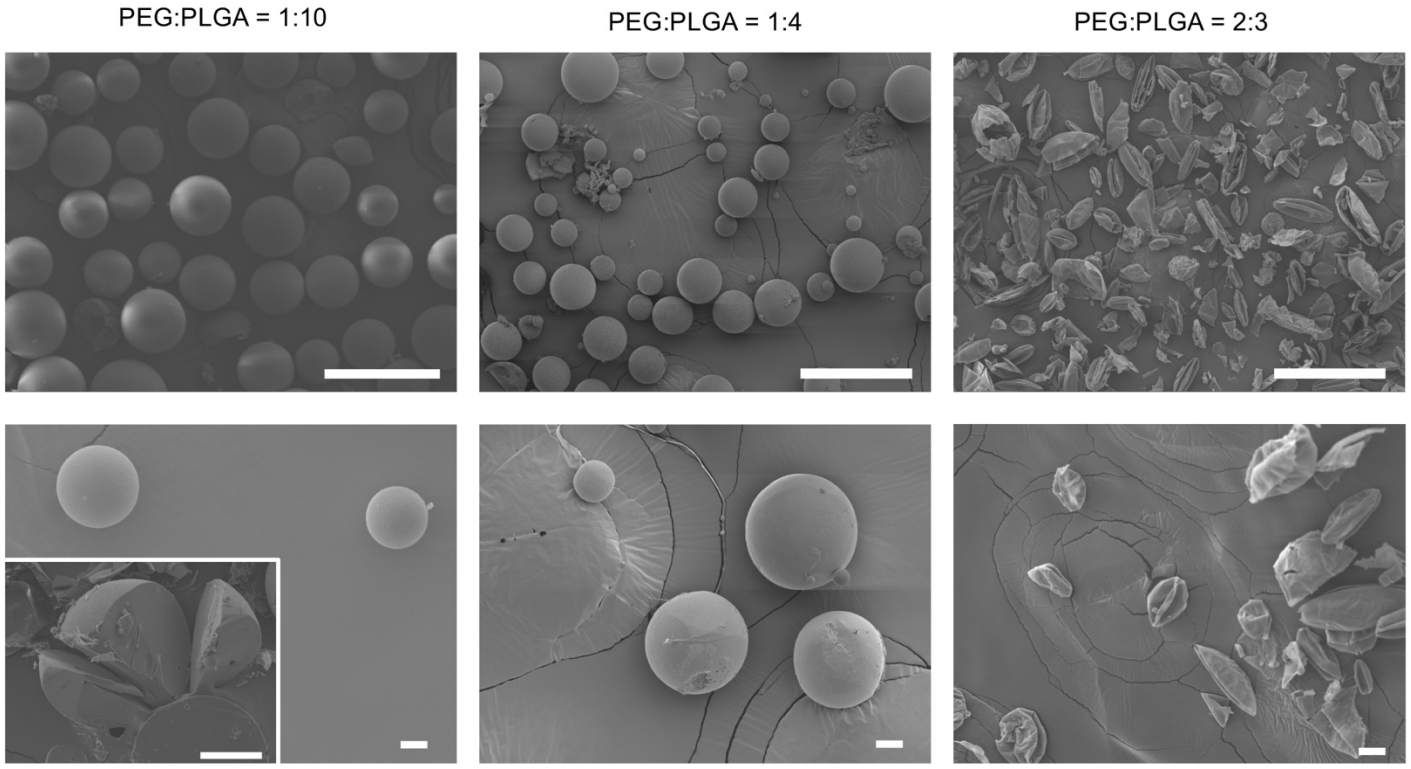
Supplementary Figure 1. Freshly isolated exosomes from primary hDPSCs and immortalized MDPC-23 cells are characterized by nanoparticle tracking analysis (A, B), demonstrating characteristic hydrodynamic diameter (d_{avg}=135 nm). Western blot analysis (C, D) of exosome-specific membrane surface markers (CD9, CD63, CD81) are abundantly present in the exosome isolate fractions but constitute only a small amount of the total cell lysate, when total protein concentrations are even, indicating that isolated extracellular vesicles are the product of the multivesicular body pathway. Freshly isolated exosomes from both cell types were labelled with DiO membrane dye, and their uptake by nascent hDPSCs was visualized by confocal microscopy (E, F; red = F-actin, blue = DAPI, green = DiO-labelled exosomes) 30 minutes after administration (scale = 15 um).

A)

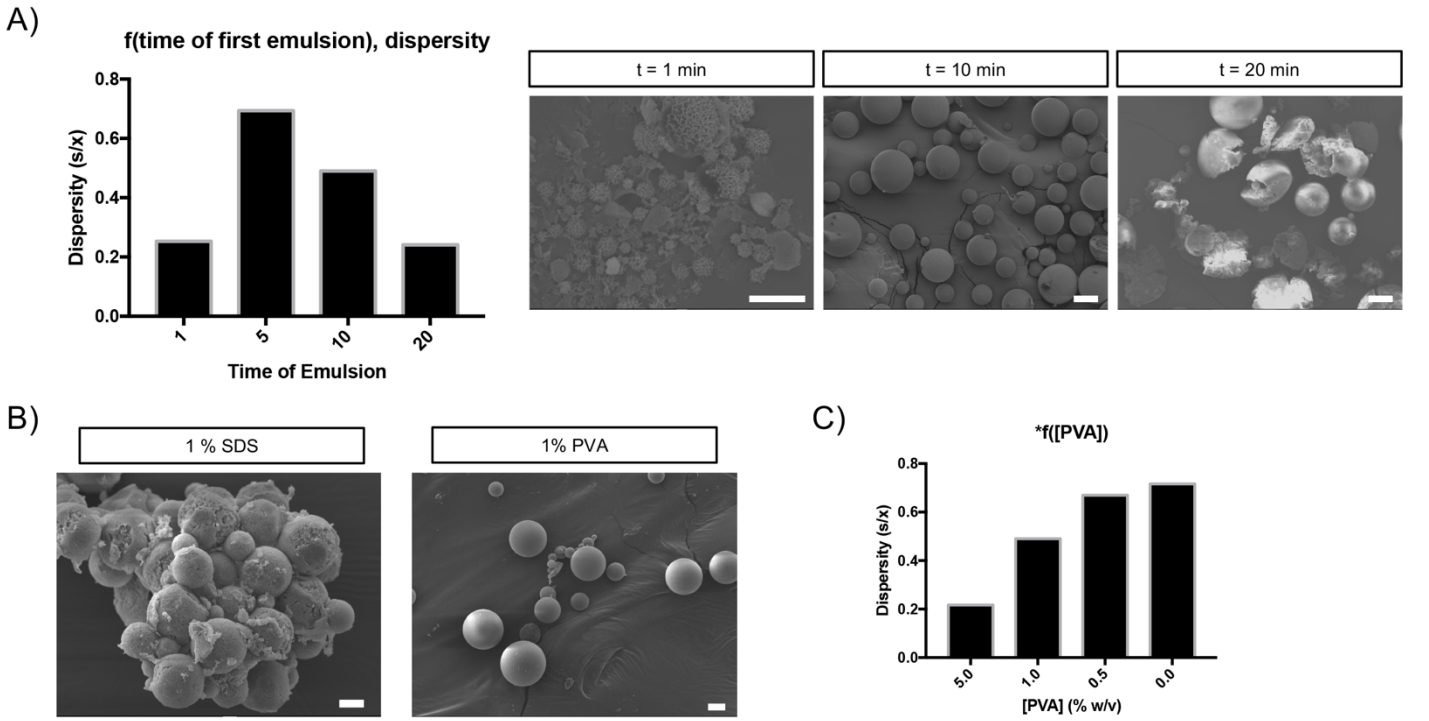
Triblock Polymer	Mn	Mw	PDI
PEG 1k-PLLA (5k)	4,531	6,567	1.4492
PEG 0.5k-PLLA (5k)	4,621	6,127	1.325
PEG 2k-PLLA (5k)	4,679	6,231	1.3370
PEG 1k-$PL_{85}G_{15}A$ (5k)	4,642	6,731	1.4498
PEG 1k-$PL_{70}G_{30}A$ (5k)	4,340	6,607	1.522
PEG 1k-$PL_{50}G_{50}A$ (5k)	4,769	6,741	1.4135
PEG 1k-PLLA (30k)	16,337	21,765	1.5896
PEG 1k-$PL_{85}G_{15}A$ (30k)	16,230	25,800	1.5897



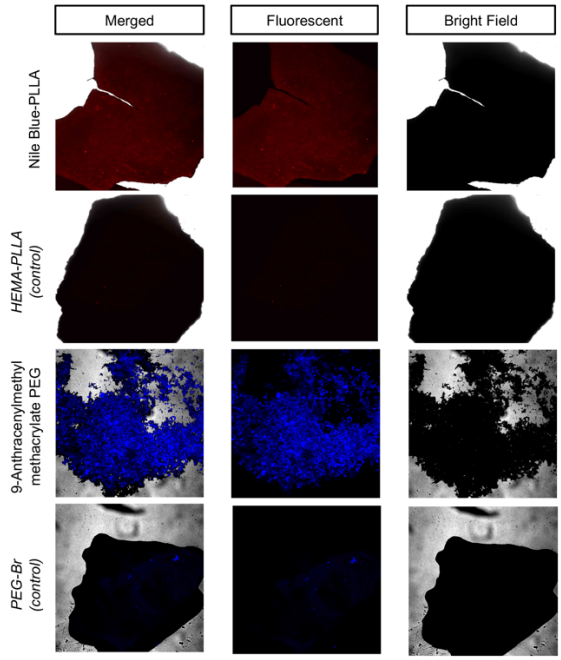
Supplementary Figure 2. Polymer characterization of PLGA-PEG-PLGA triblock copolymer. Molecular weight is determined by gel permeation chromatography (A); chemical composition and purity is assessed by ^{1}H and ^{13}C NMR (B, C, D).



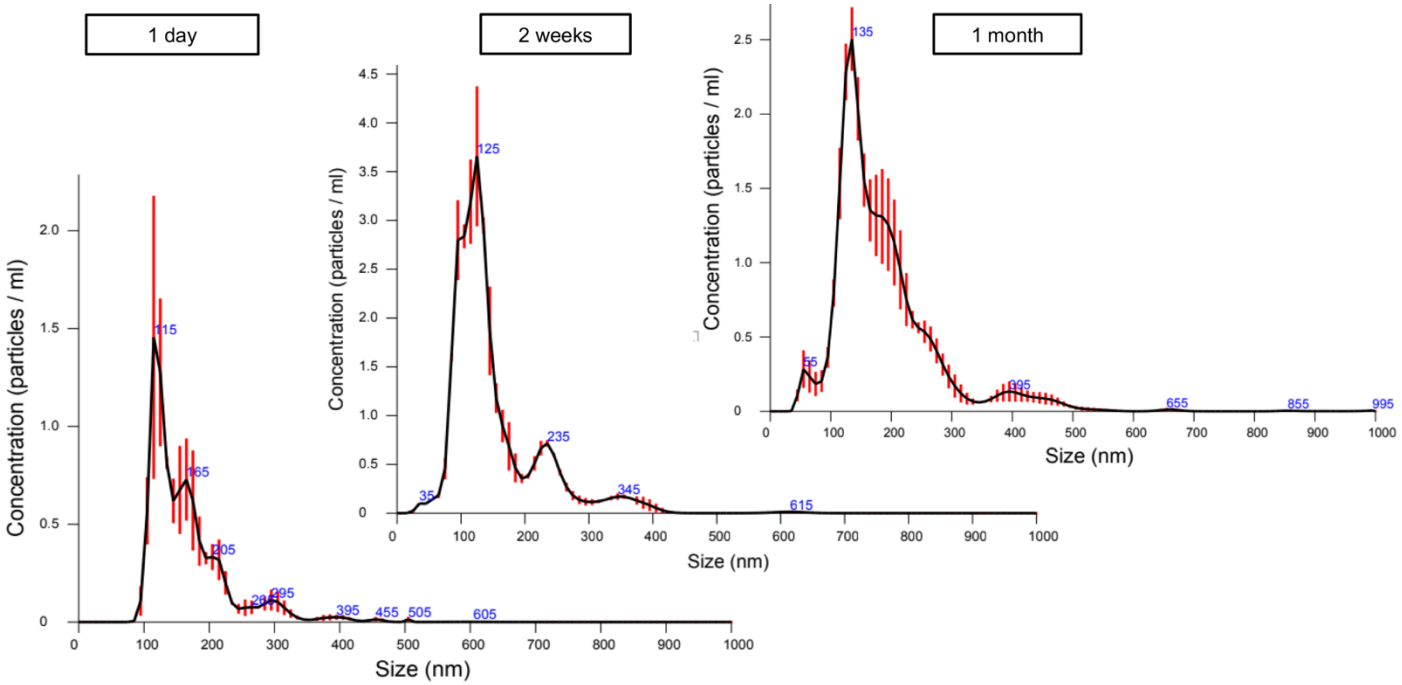
Supplementary Figure 3. The relative ratios of PEG and PLGA are critical design parameters of the PLGA-PEG-PLGA triblock copolymer. Top, scale = 100 μm; lower, scale = 10 μm.



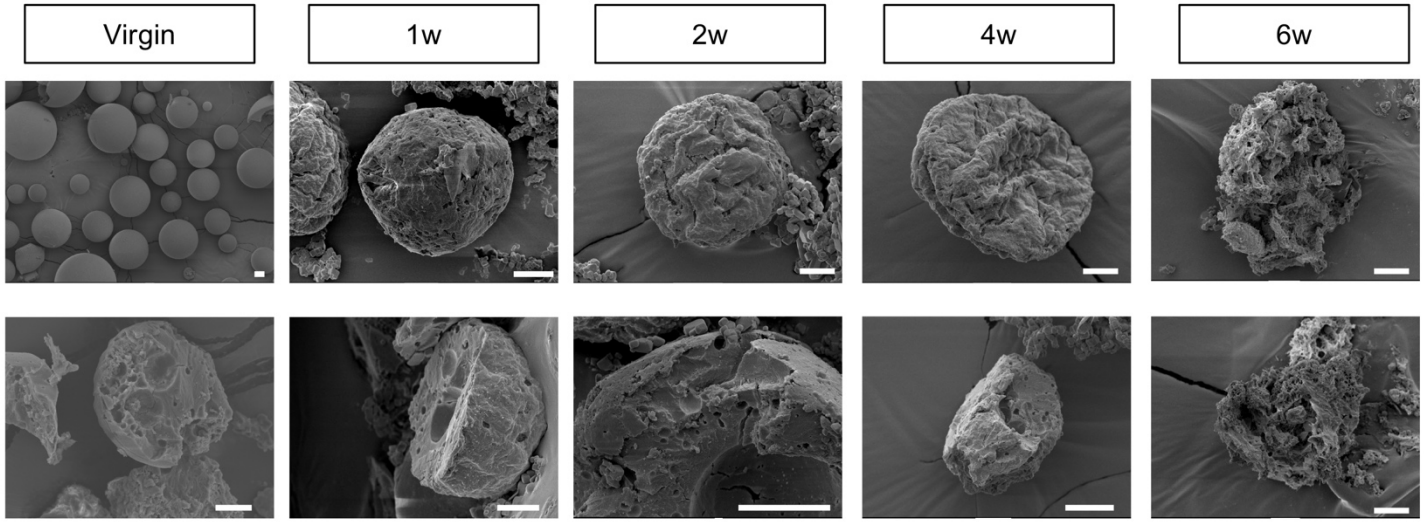
Supplementary Figure 4. EXO-MS morphology is a function of fabrication parameters. Particle dispersity decreases with increasing time of first emulsion, while average particle size increases with increasing time of first emulsion under the same second emulsion conditions (A). Polyvinyl alcohol (PVA) surfactant in the second emulsion results in smoother, more uniform particles than sodium dodecyl sulfate surfactant (B). Increasing PVA concentration leads to narrower particle dispersities, however too high of PVA concentration may render the particles cytotoxic and is difficult to remove completely by washing. Scale = 20 um.



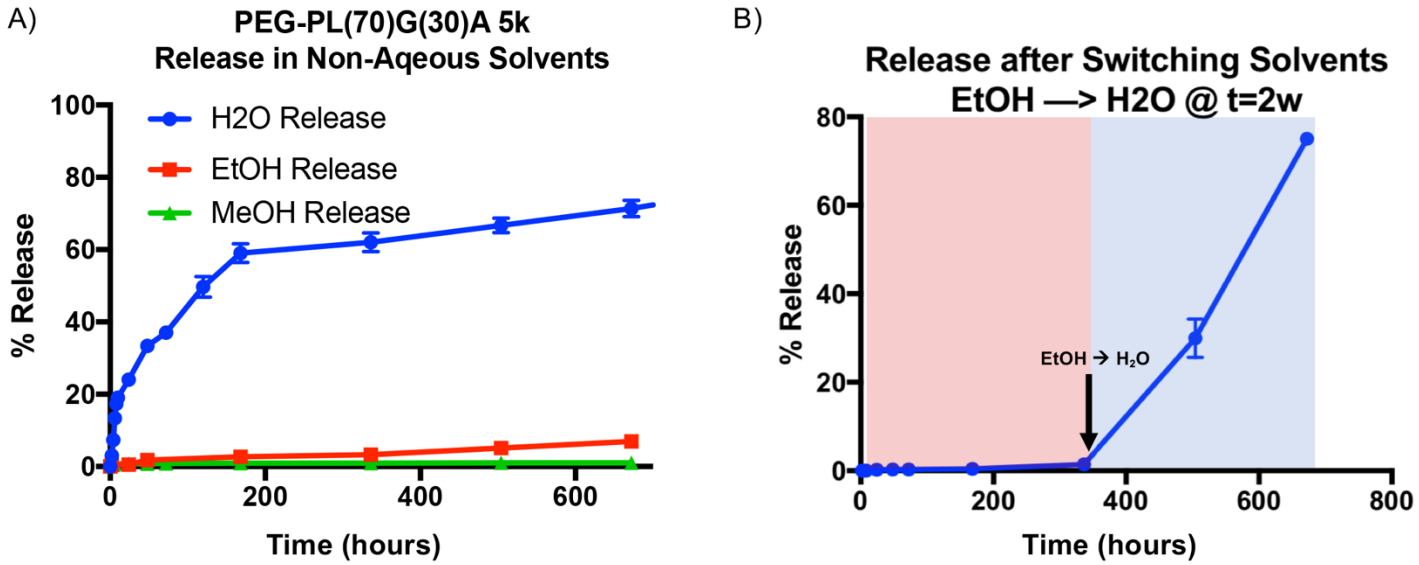
Supplementary Figure 5. Fluorescence of labelled polymer segments visualized by laser confocal microscopy, compared to nonfunctionalized precursor polymers.



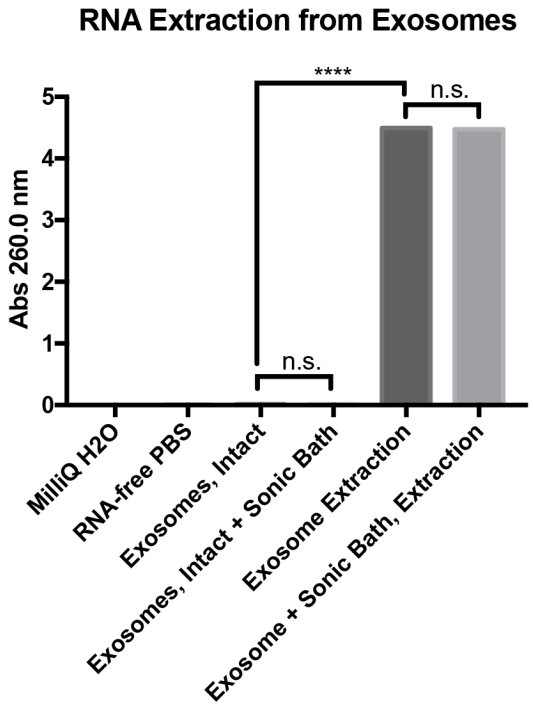
Supplementary Figure 6. Nanotracking analysis size distribution results at prescribed release time points: 1 day, 2 weeks, and 1 month. At each time point, DPSC-EXO maintain their characteristic size and dispersity, indicating that they are not physically modified as a result of encapsulation or release.



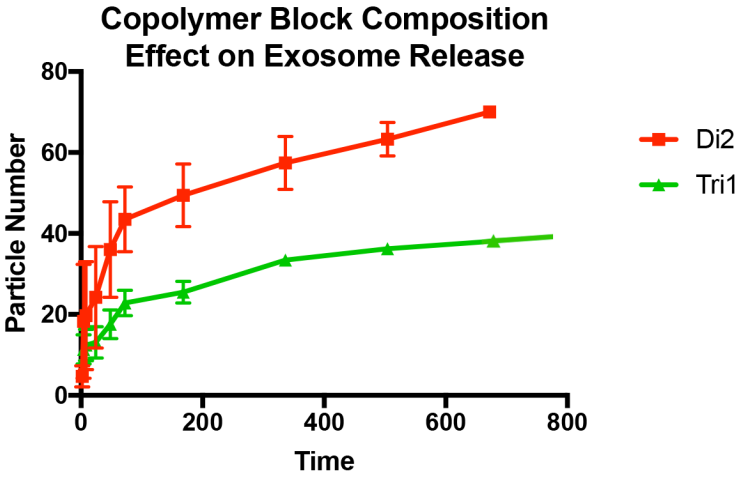
Supplementary Figure 7. SEM micrographs of EXO-MS at different degradation stages: virgin, 1 week, 2 weeks, 4 weeks, and 6 weeks. Images in the top row show changes to gross particle morphology; images in the bottom row show changes to the internal particle morphology throughout degradation. Scale = 10 μm.



Supplementary Figure 8. EXO-MS were incubated in water, ethanol, and methanol, and the release of DPSC-EXO was measured by nanotracking analysis (A). Negligible amounts of DPSC-EXO were released in either methanol or ethanol, indicating that the solvent-wetting procedure to increase surface hydrophilicity did not cause significant amounts of DPSC-EXO load to be released before implantation. Particles incubated in ethanol for two weeks then switched to water began releasing DPSC-EXO immediately after the solvent change and resumed a normal release profile (B).



Supplementary Figure 9. Validation of phenol-based exosome RNA extraction method; RNA is only detected when the exosome membrane is lysed, while intact exosomes yield no detectible RNA.



Supplementary Figure 10. EXO-MS fabrication parameters affect particle morphology and resulting release profile of DPSC-EXO (Tri1: PLGA-PEG-PLGA EXO-MS; Di2: PLGA-PEG EXO-MS).