Electronic Supplementary Material (ESI) for Biomaterials Science.

Vaccine delivery by zwitterionic polysaccharide-based hydrogel microparticles showing enhanced immunogenicity and suppressed foreign body responses

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Keywords: hydrogel; vaccine; immunotherapy; zwitterionic polymer; polysaccharides

Supplementary Information

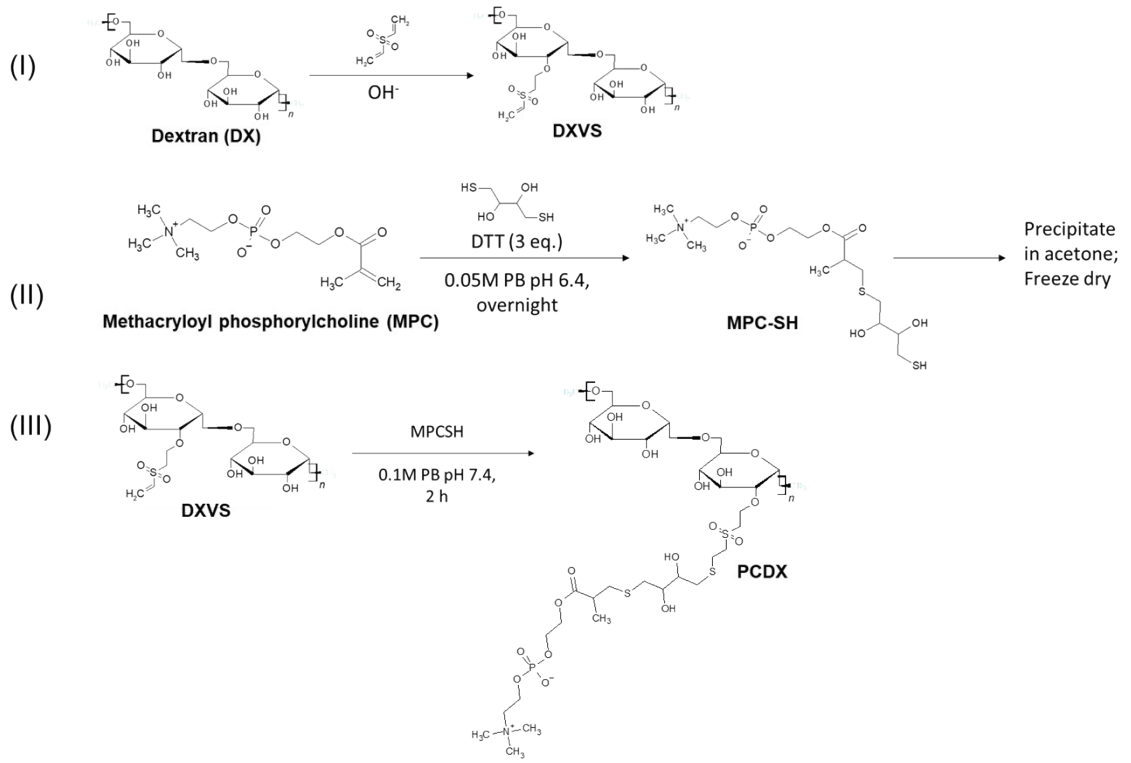


Figure S1. The synthesis scheme of DXVS and zwitterionic PCDX. (I) DX was activated with vinyl sulfone group (VS) by reacting DX at 5% wt/V polymer concentration with DVS, at molar ratio DVS:OH of 1.2:1. Reaction occurred in 0.02M NaOH and stopped with 0.02M HCl after various time points to control degree of modifications (DM). **(II)** MPC-SH was synthesized by reacting MPC to DTT at molar ratio MPC:DTT of 1:6 in 0.05M phosphate buffer, pH 6.4 overnight at room temperature. The product was extracted via acetone precipitation. **(III)** PC was synthesized by reacting MPC-SH with DXVS at molar ratio VS:SH of 1:3 in 0.1M phosphate buffer of pH 7.4 for 2 hour at room temperature.

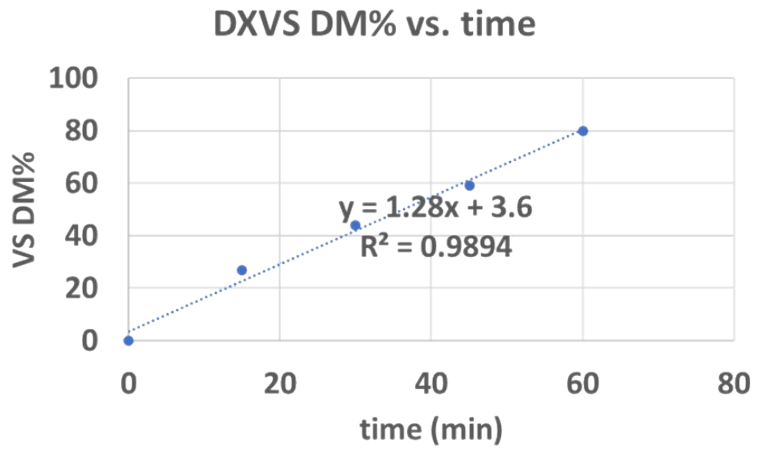
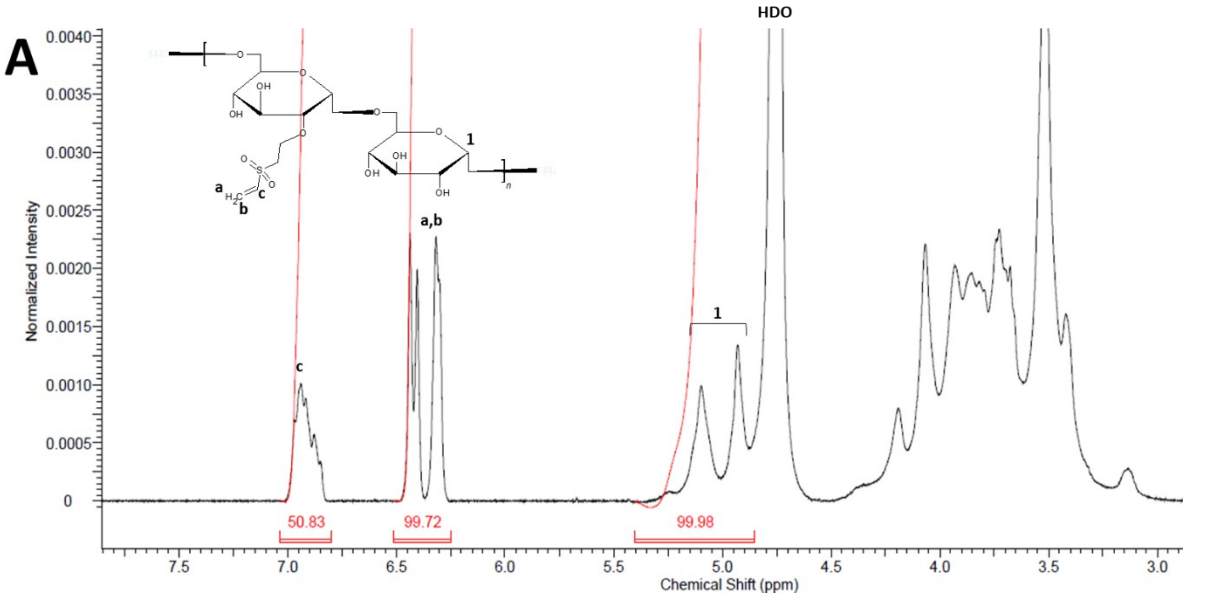
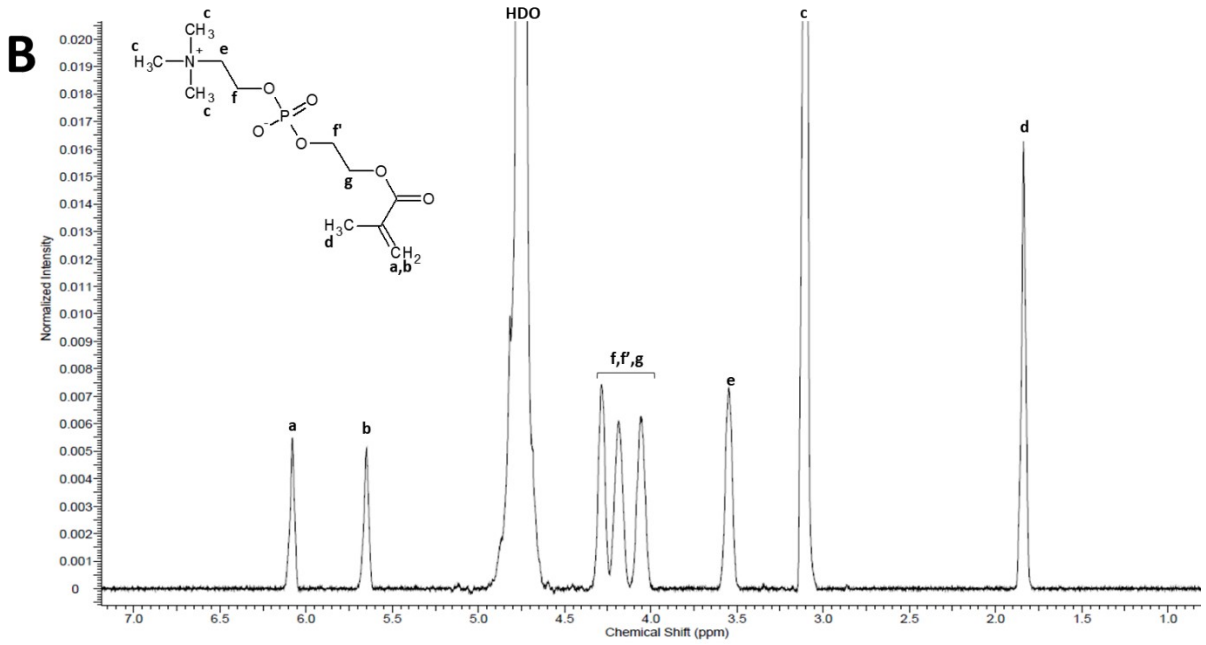


Figure S2. The relationship between the DM of VS on DX with time at 5% wt/V DX polymer concentration and 0.02M NaOH. Reactions were stopped with 0.02M HCl.


A
HDO
Normalized Intensity
Chemical Shift (ppm)
a,b
c
1
50.83
99.72
99.98


B
HDO
Normalized Intensity
Chemical Shift (ppm)
a
b
c
d
e
f,f',g

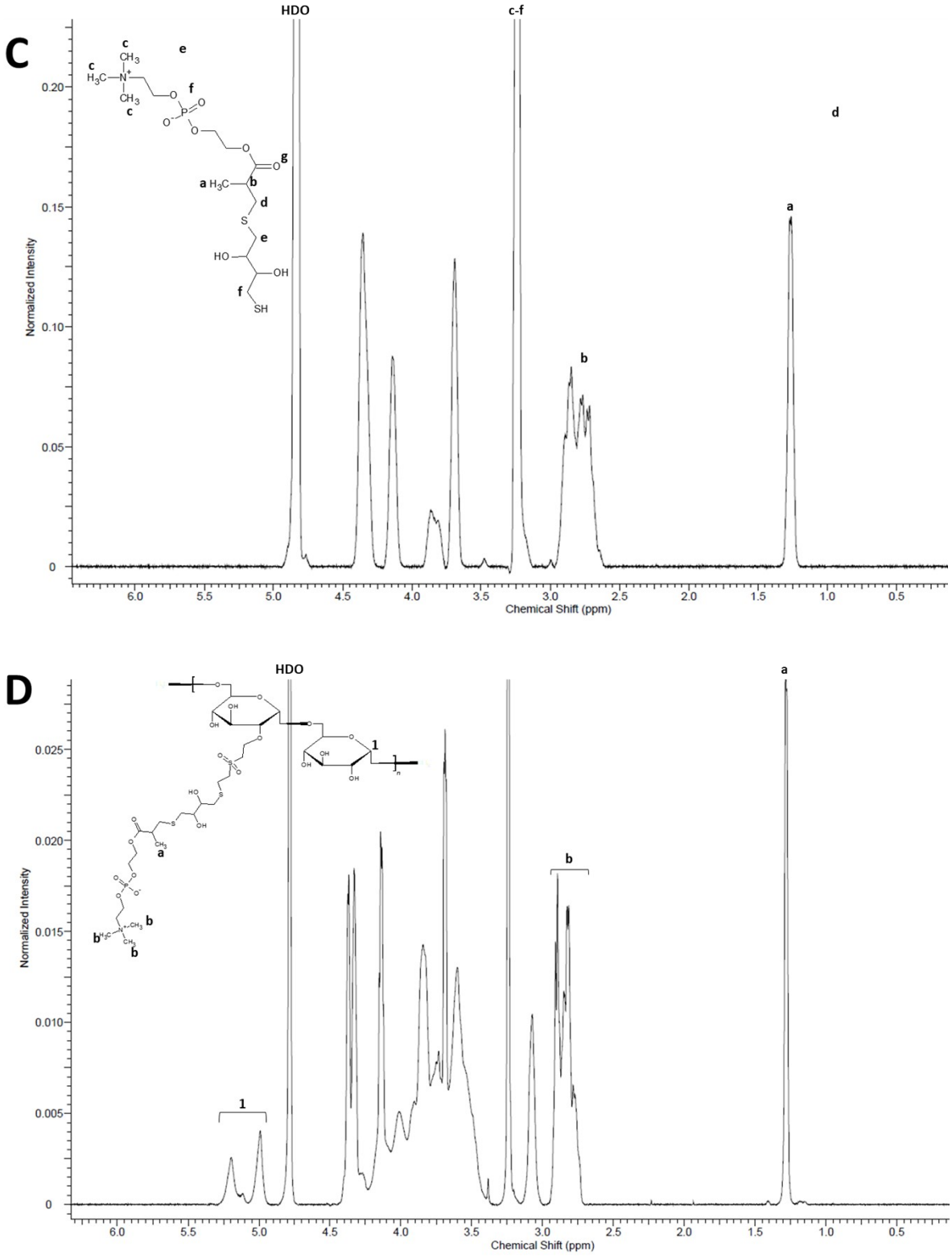


Figure S3. The chemical structure and ^{1}H-NMR spectra of A) VS functionalized DX, **B)** MPC, **C)** thiolated MPC, MPC-SH and **D)** DX modified with PC.

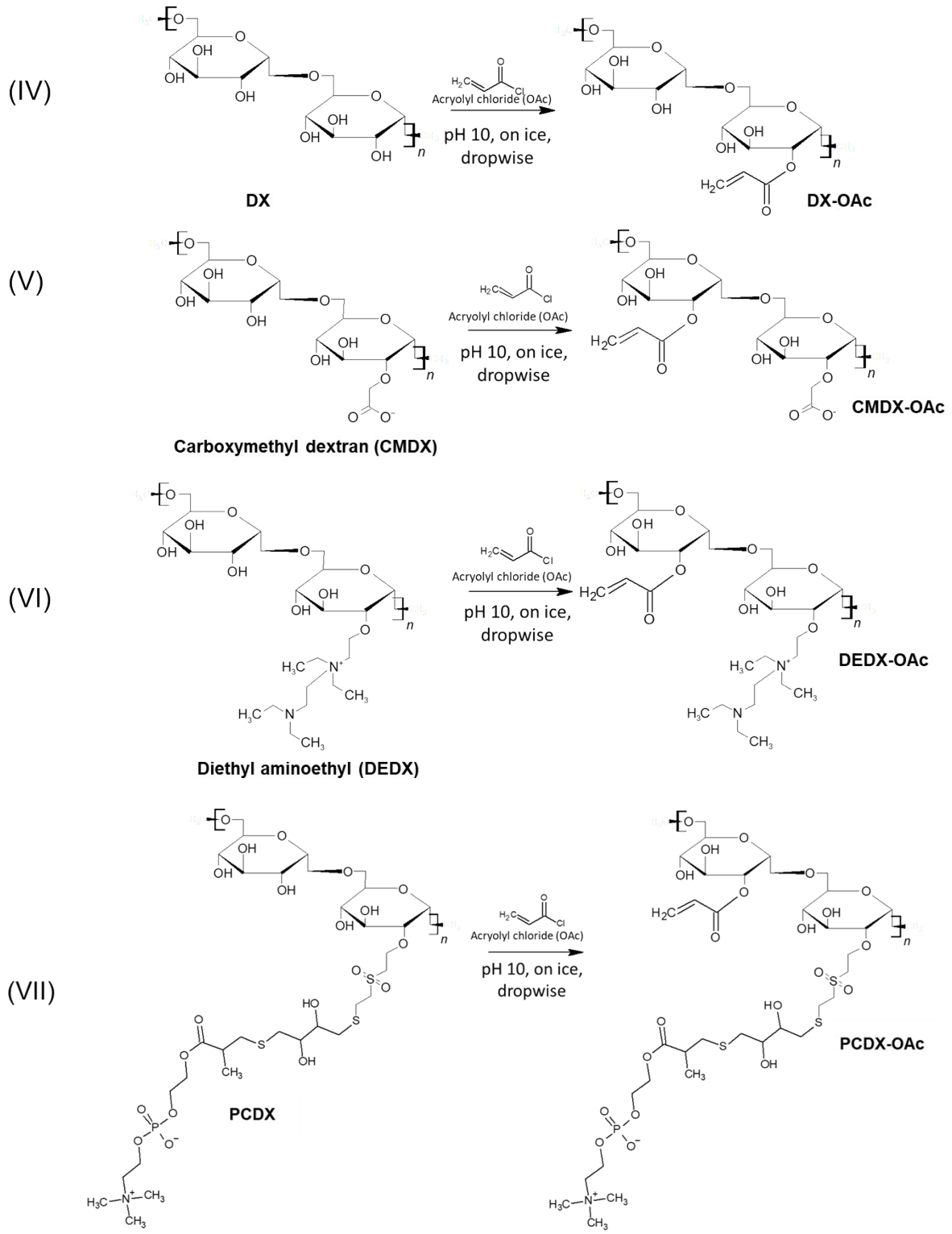
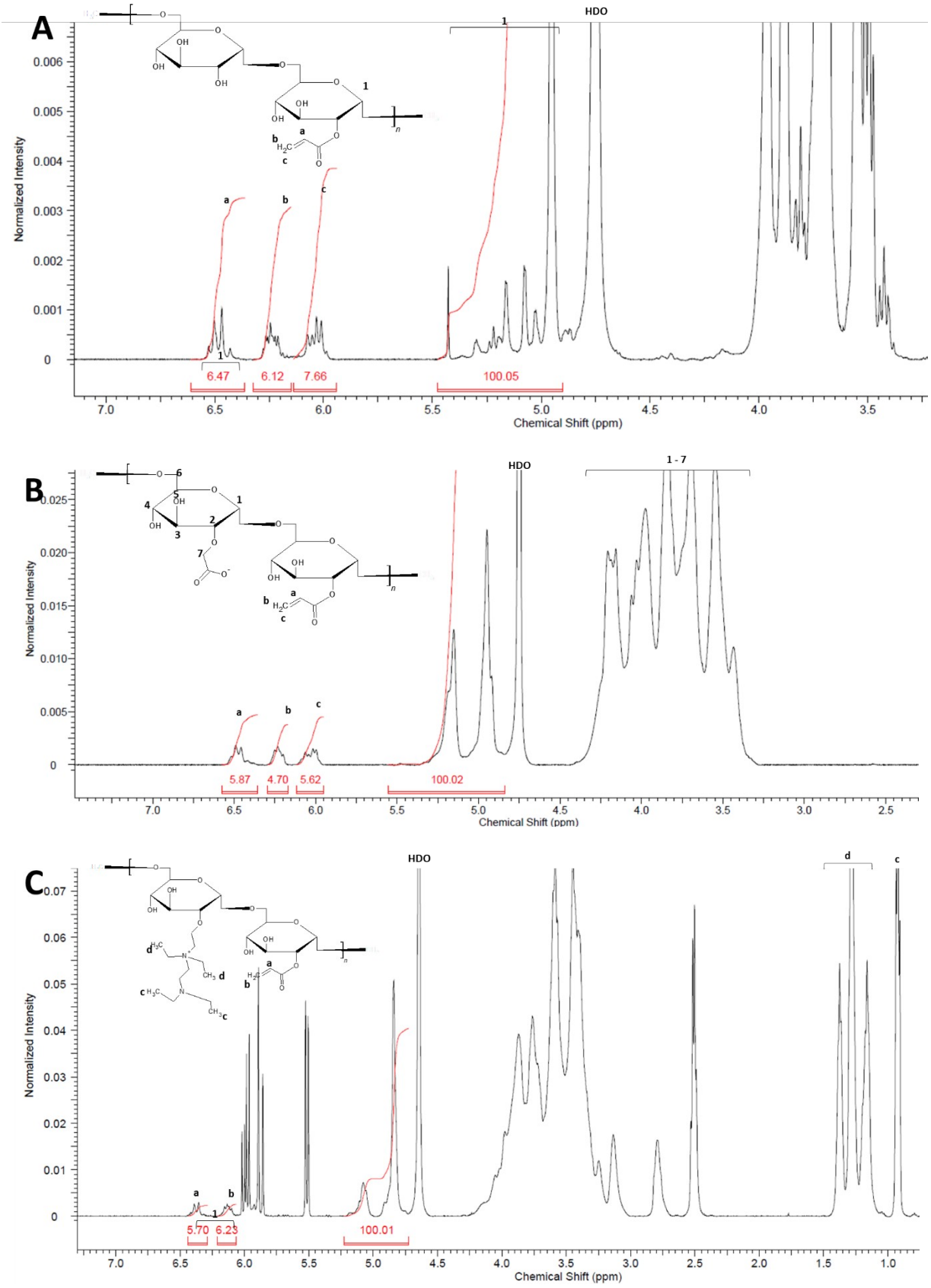


Figure S4. The synthesis scheme -OAc functionalized DX and charge derivatives. (IV-VII) DX and charge derivatives, CMDX, DEDX and PCDX were activated with acryloyl group (-OAc) by reacting polysaccharides at 5% wt/V polymer concentration with acryloyl chloride, at a molar ratio OAc:OH of 0.5:1 in ddH_2O. 5M NaOH was added dropwise to maintain reaction pH at 9 to 10 for 1 hour, in ice bath. Reaction was stopped with 6M HCl addition to pH 5. All -OAc functionalized products were purified with dialysis and lyophilized.


A
HDO
1
a
b
c
6.47
6.12
7.66
100.05
Normalized Intensity
Chemical Shift (ppm)
B
HDO
1 - 7
a
b
c
5.87
4.70
5.62
100.02
Normalized Intensity
Chemical Shift (ppm)
C
HDO
d
c
a
b
5.70
6.23
100.01
Normalized Intensity
Chemical Shift (ppm)

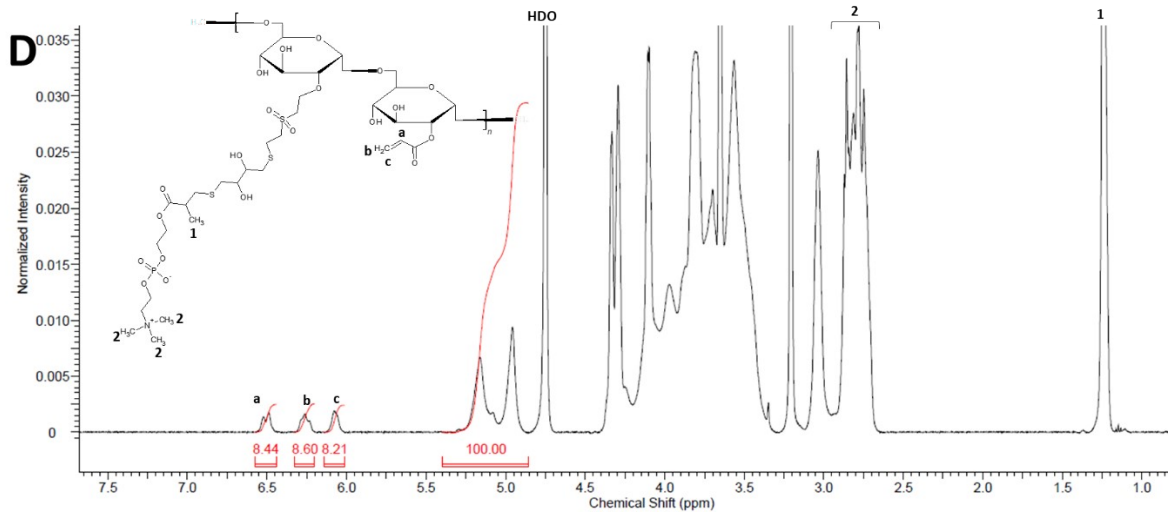


Figure S5. The chemical structure and ^{1}H-NMR spectra of -OAc functionalized A) dextran (DX-OAc), **B)** carboxymethyl dextran (CMDX-OAc), **C)** diethyl aminoethyl dextran (DEDX-OAc) and **D)** phosphorylcholine-decorated dextran (PCDX-OAc).

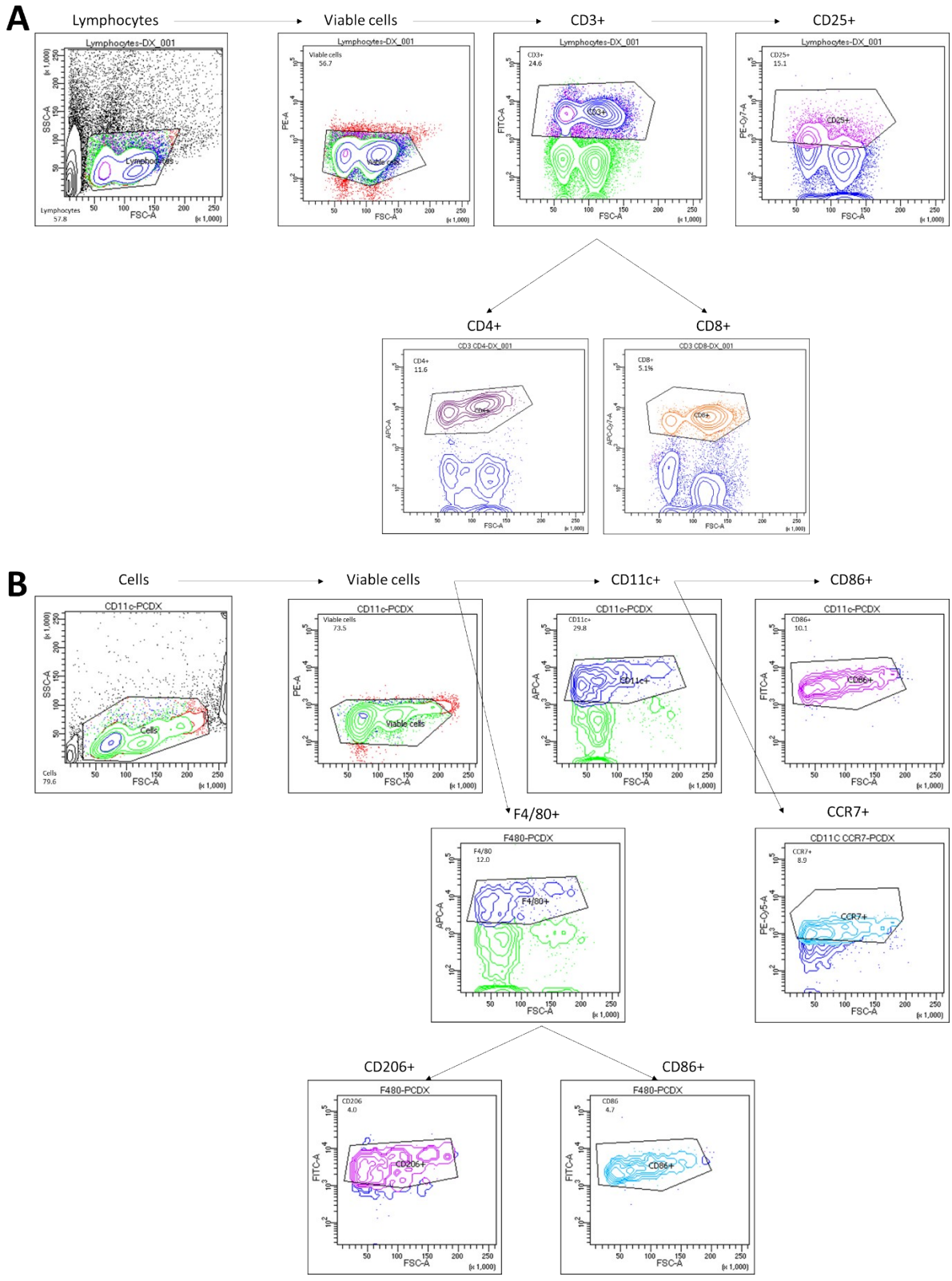


Figure S6. Representative flow cytometry flow from C57BL\6 mice tissue samples post hydrogel injection, showing gating strategy for A) CD3+, CD4+, CD8+ T cell subsets and B) CD11c+ with activation markers CD86 and CCR7, as well as F4/80+ cells with CD206 and CD86, %total of each subsets are labelled in the graphs.

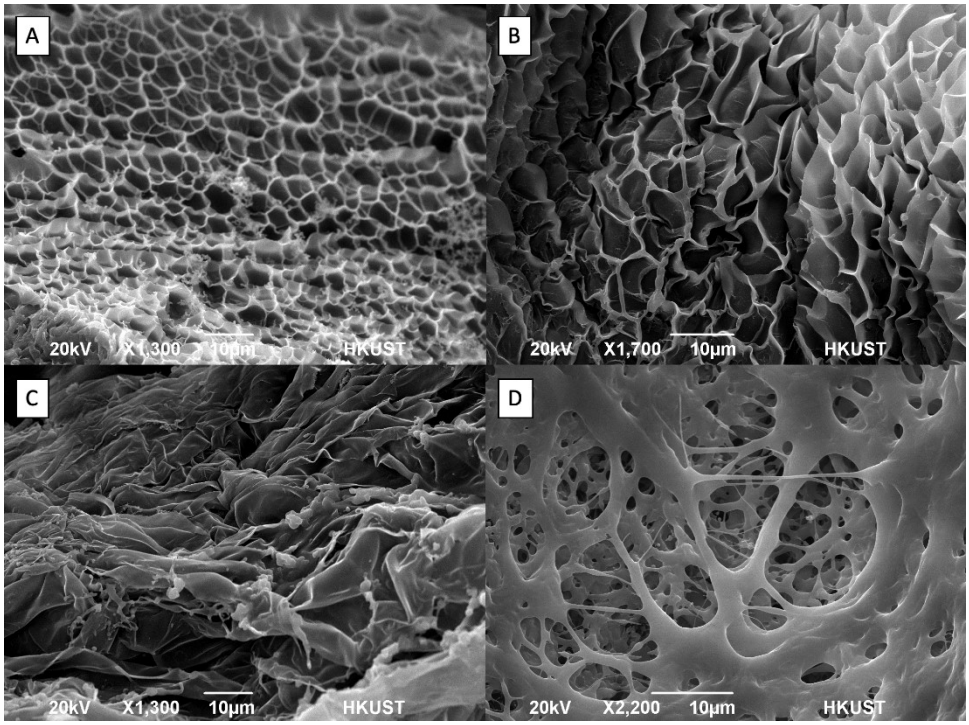


Figure S7. Scanning electron microscope images of A) DX, B) DEDX, C) CMDX and D) PCDX. Bulk hydrogels were prepared with 40% wt/V DX-OAc, DEDX-OAc, CMDX-OAc and PCDX-OAc and crosslinked with DTT. Hydrogels were incubated overnight at 37℃, in humidified chamber to ensure complete gelation. Subsequently, hydrogels were allowed to swell in ddH_2O for 2 hr. The hydrogels were blotted dry with Kimwipe and compaction with centrifuge, 1000 rpm for 30 sec. Subsequently, hydrogels were subjected to snap freezing in liquid N_2 and lyophilization. The freeze dried hydrogels were cut into smaller pieces of ~ 2 mm and subjected to gold sputter coating (K575xd, Emitech Ltd). The hydrogels were imaged with scanning electron microscope (JSM-7100F JEOL) at 20 kV.

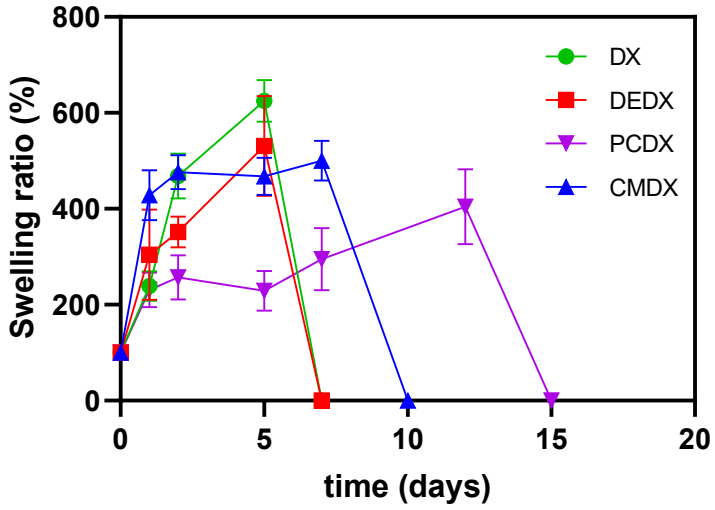


Figure S8. The swelling behavior of degradable -OAc modified DX, CMDX, DEDX and PCDX bulk hydrogels.

Table S1. Degree of modifications (DM) of -OAc on hydrogel precursors, estimated from ^{1}H-NMR spectra

Hydrogel precursors	-OAc DM(%)
DX-OAc	6.75
CMDX-OAc	5.43
DEDX-OAc	6.05
PCDX-OAc	8.42

Table S2. The average endotoxin level released from hydrogel washed with PBS after 24 hours incubation.

Hydrogels	Average Endotoxin Level (EU/mL)
CM	0.165 ± 0.045
DE	0.109 ± 0.015
DX	0.125 ± 0.004
PC	0.197 ± 0.002
PBS	0.0128 ± 0.004