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

Supporting Information

Sulfonated, oxidized pectin-based double crosslinked bioprosthetic valve leaflets for synergistically enhancing hemocompatibility, cytocompatibility and reducing calcification

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1. Experimental section

1.1 Preparation of double-modified anticoagulant HepLBm, SAP

1.1.1 Cytotoxicity of double modified pectin

Mouse fibroblast cells (L929) and human umbilical vein endothelial cells (HUVECs) were used to evaluate the cytotoxicity of SAP in vitro. Different concentrations of SAP were added to the cell culture medium containing adherent L929 cells or HUVECs (1×10^4 cells/well). After the preset time was reached, no serum culture medium containing 10% Cell Counting Kit-8 was added to each well, and cells were incubated in the dark for an hour. 140 μL supernatant was used for detecting the optical density at 450 nm with a microplate reader (SAF-680 T). Experiments were made in sextuplicate.

2.2. Hemocompatibility

2.2.1 Protein adsorption

The PP samples rinsed with PBS buffer were placed in a cell-cultured plate and incubated with BSA-FITC (4 mg/mL) and FBG-FITC (0.2 mg/mL) respectively at 37°C for 2h. After rinsing three times, the morphology of the PP sample was observed using a fluorescence microscope (OLYMPUS IX3P1F, Japan).

1.3 Cytotoxicity of PP samples

The cytotoxicity of PPs samples was assessed through the extraction method described in ISO 10993-5. Briefly, the tailor PP samples were sterilized with 75% ethanol for 24 h, then followed by rinsing thoroughly with sterile PBS buffer. And, the treated PP samples were immersed into the cell culture medium and incubated at 37°C for 3 days to obtain the sample extract. HUVECs at the density of 10000 cells/well were seeded in 96-well plates for 24 h. Then, after discarding the old medium, 150 μL extract was located in each well, where the cells were cultured for another 72 h. CCK-8 kit was used to determine the cytotoxicity of PP samples according to the supplier's instructions.

2. Supporting figure

Sample	C%	N%	H%	S%	DS
Heparin	19.43	2.55	8.76	11.03	1.28
SAP	24.35	3.55	8.23	12.66	1.17
SP	31.95	4.58	8.04	16.33	1.15
Pectin	38.65	0.14	6.89	0.20	--

Figure S1. EA data and the calculated sulfation degrees (DS, sulfonic acid groups per uronic acid) values for heparin, SAP, SP, and pectin.

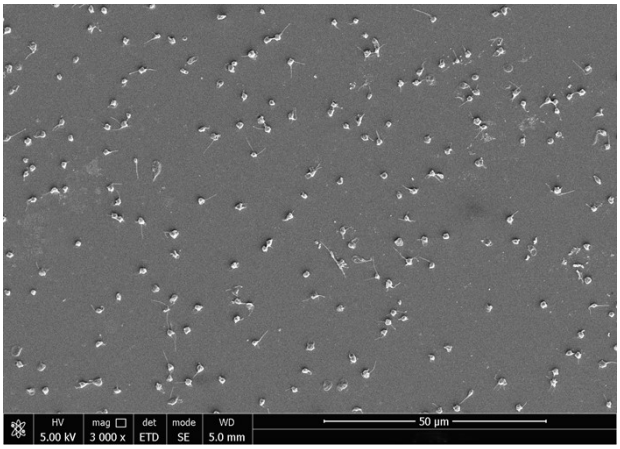

Figure S2. SEM image of static platelet adhesion on the surface of AP-coated PVA plate.

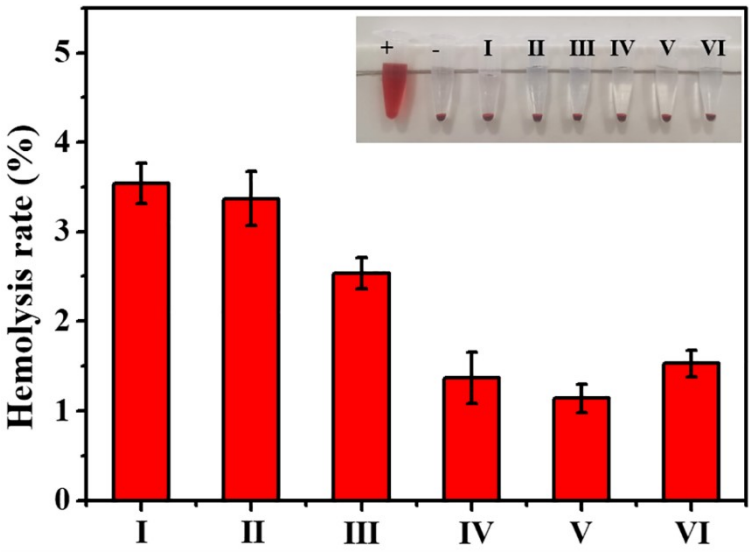

Figure S3. Hemolysis ratios of RBCs incubated with SAP, and heparin. The insert images were physical diagram of hemolysis (Ⅰ: 0.15 mg/mL heparin, Ⅱ: 0.55 mg/mL heparin, Ⅲ: 1.15 mg/mL heparin, Ⅳ: 0.15 mg/mL SAP, Ⅴ: 0.55 mg/mL SAP, Ⅵ: 1.15 mg/mL SAP). (+) represents positive control, and (−) represents negative control.

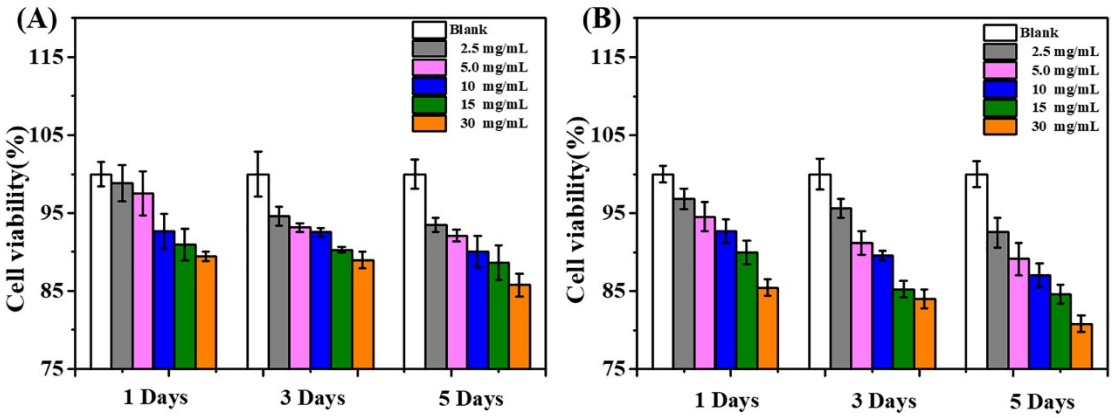

Figure S4. Cell viability of (A)HUVECs and (B) L929 in different extractions of SAP (n=6).

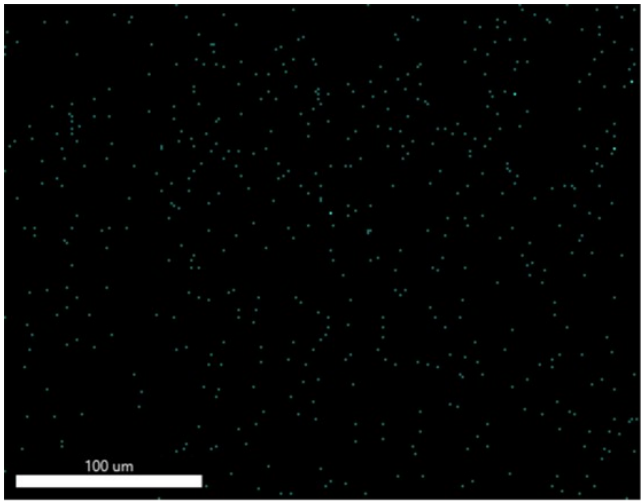

Figure S5. The sulfur distribution on the surface of AP-PP samples determined by EDS mapping.

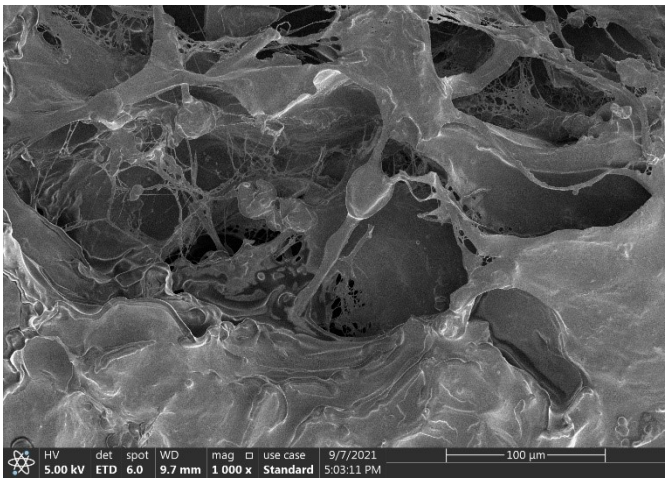

Figure S6. SEM images of Decellularized-PP sample.

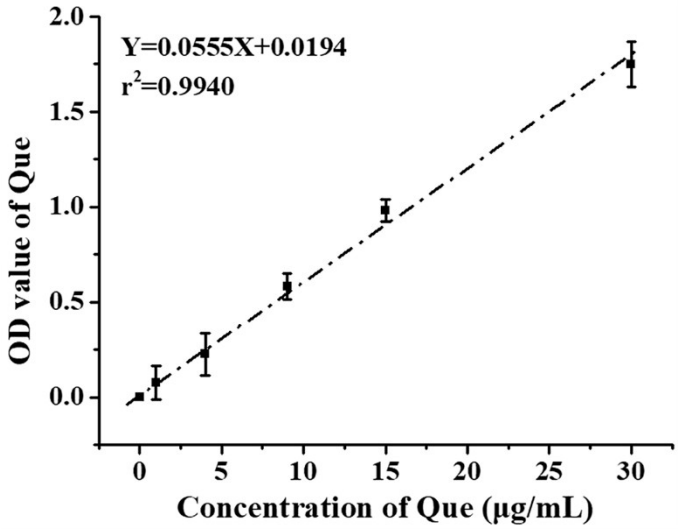

Figure S7. Standard optical-to-concentration curve of different concentrations of quercetin.

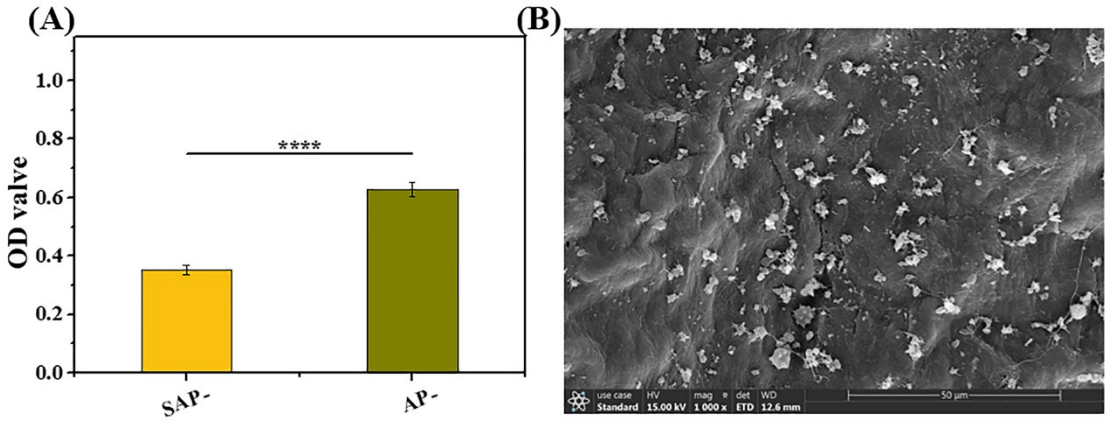

Figure S8. (A) LDH quantification of adhered platelets on the surface of SAP-PP and AP-PP, and (B) SEM images of adhered platelets on the surface of AP-PP samples.

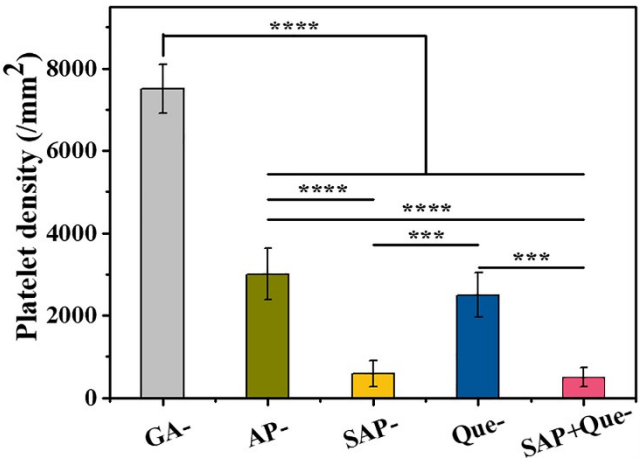

Figure S9. The density of adhered platelets on PP sample surfaces (n = 6).

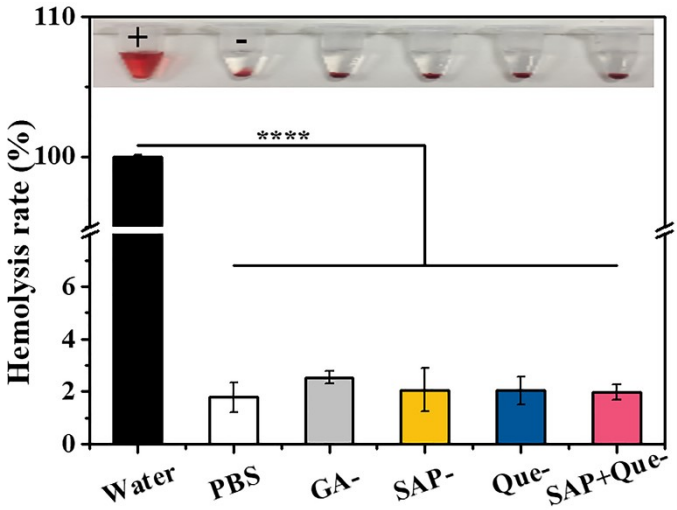

Figure S10. Hemolysis ratios of the crosslinked PP samples (the inserts were the physical maps).

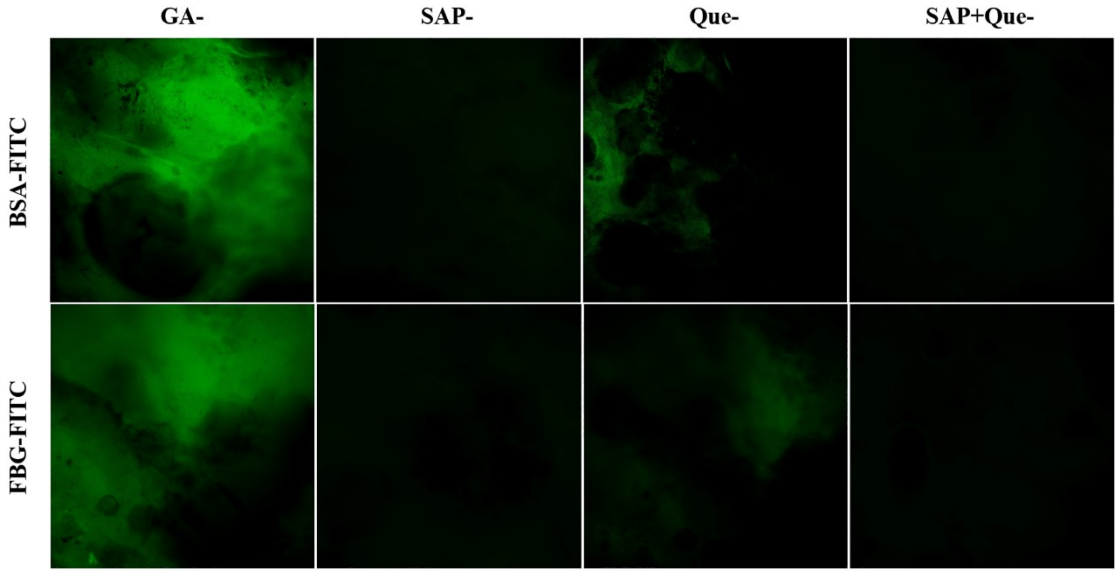

Figure S11. Fluorescence microscope images of adsorbed BSA-FITC and FBG-FITC on the PP samples (bar =200 μm).

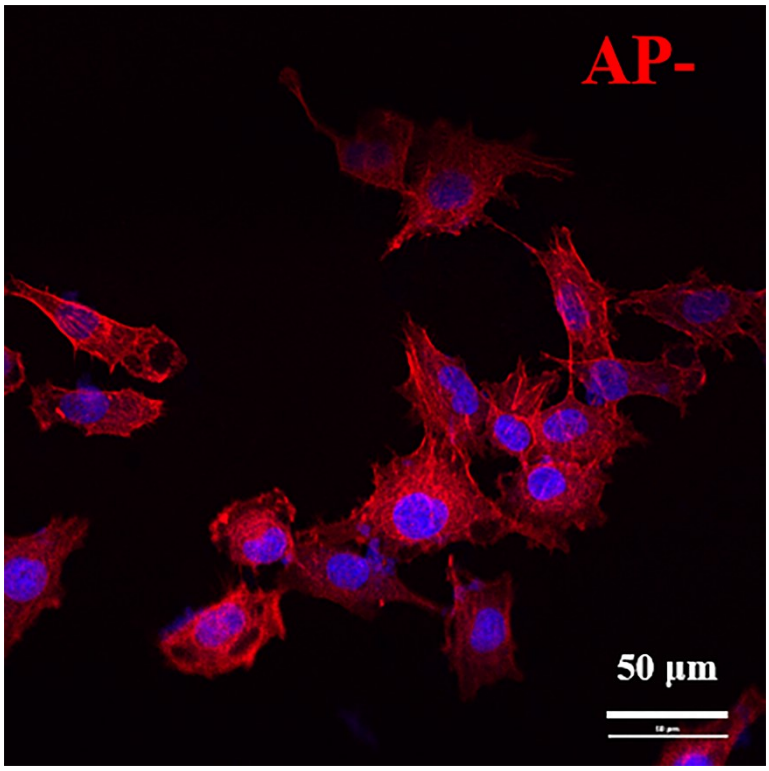

Figure S12. HUVECs stained by TRITC-phalloidin (red) and DAPI (blue) after 3-day cultivation on the surface of AP-PP (bar = 50 μm).

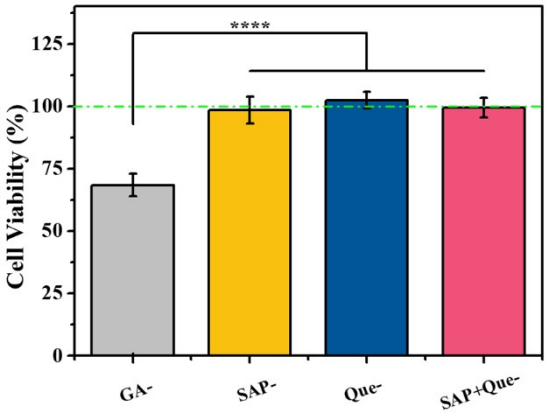

Figure S13. Cell viability of HUVECs in different extractions (n = 6).

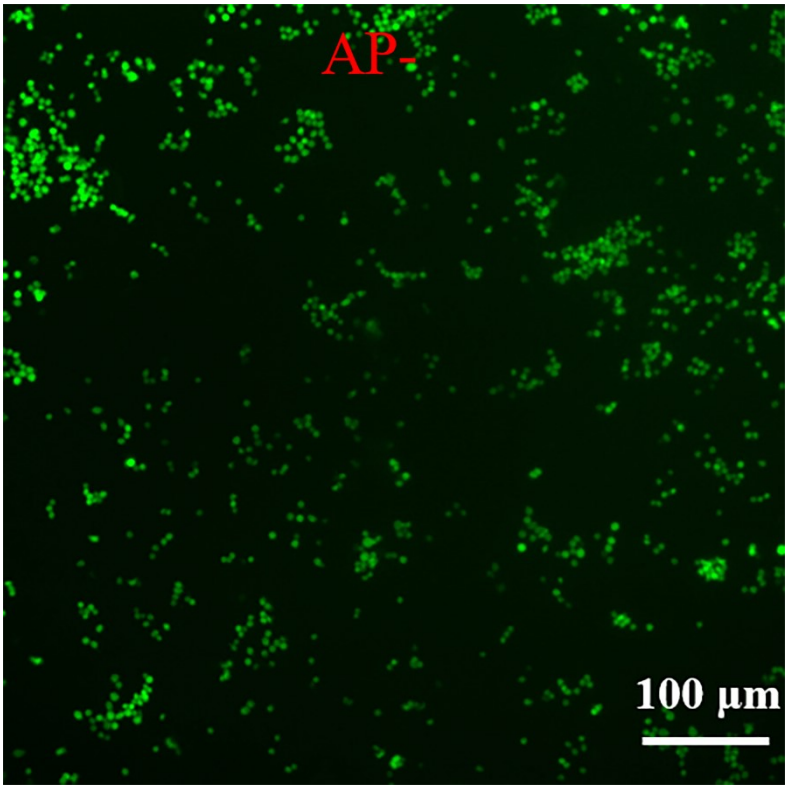

Figure S14. Live-cell staining of RAW264.7 adhered to the surface of AP-PP after culturing for 3 days.