Electronic Supplementary Material (ESI) for Biomaterials Science.
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Supplementary data:

Electron beam irradiation modified carboxymethyl chitin microsphere-based hemostatic material with strong blood cell adsorption for hemorrhage control

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Methods

^{1}H-NMR and FTIR

The ^{1}H-NMR spectrum of CMCH was determined with a Mercury VX-300 spectrometer after degradation with 20% DCl at 60 °C for 24 h. The FTIR spectra of the microspheres were tested with PerkinElmer Spectrum One FTIR spectrophotometer (Waltham, USA).

Cytotoxicity assay

Twenty milligrams of the microsphere sample were put into a tube with the medium at 37 °C for 24 h to obtain the supernatant as the conditioned medium. Two hundred microliters of cell suspension (3×10^3 NIH 3T3 cells) were transferred to the wells and incubated for 12 h in a 37 °C, 5% CO_2 cell incubator. 150 μL complete medium and 50 μL of the above-conditioned medium were added to replace the original medium. 20 μL of CCK-8 reagent was added and incubated for 2 h after 24 and 48 h co-cultivation. The optical density value of each well was measured by a SpectraMax® 190 microplate reader at 450 nm (Molecular Devices, USA).

In vivo degradation

The SD rats (~250 g) were anesthetized by intraperitoneal injection of 10% chloral hydrate. The sterile CCM-g-AA@DA microspheres suspension (0.4 mL of 20 mg/mL) was injected into its subcutaneous tissue in the upper back through a 22 G needle. At predetermined time points, the tissue surrounding the implant was taken by scissors, and the remaining microspheres were checked and photographed. Each time point has 4 degradation sites.

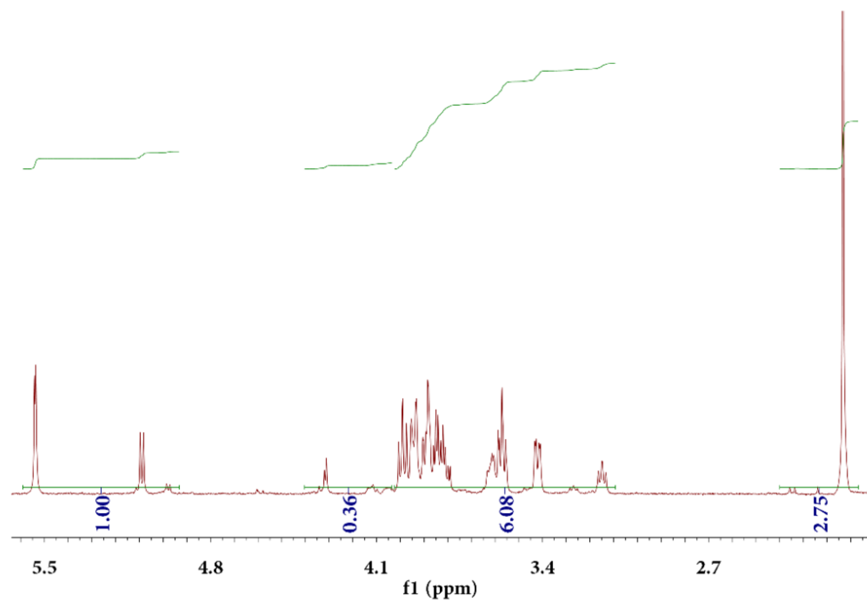

Fig. S1 ^{1}H-NMR spectrum of CMCH in 20% DCl/D_2O.

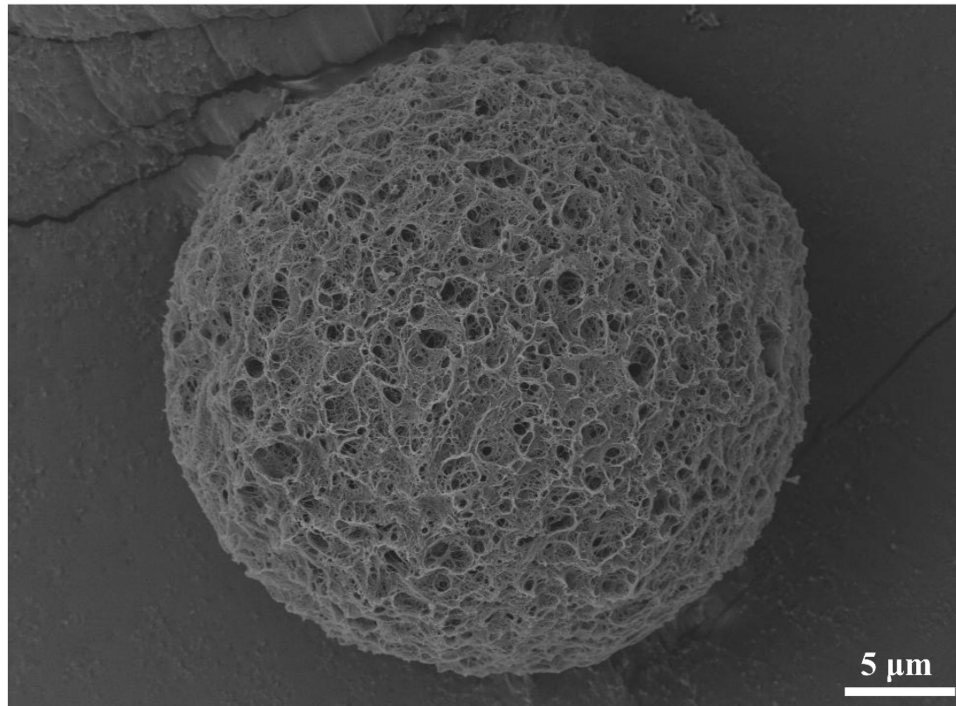

Fig. S2 SEM image of the porous carboxymethyl chitin microsphere CCM. Scale bar: 5 μm.

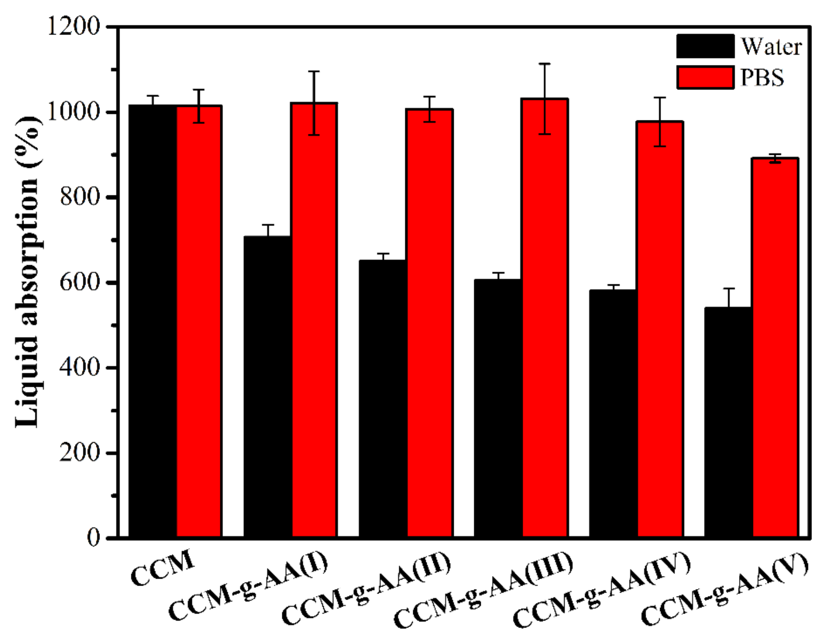

Fig. S3 Liquid absorption (30 s) of CCM-g-AA microspheres.

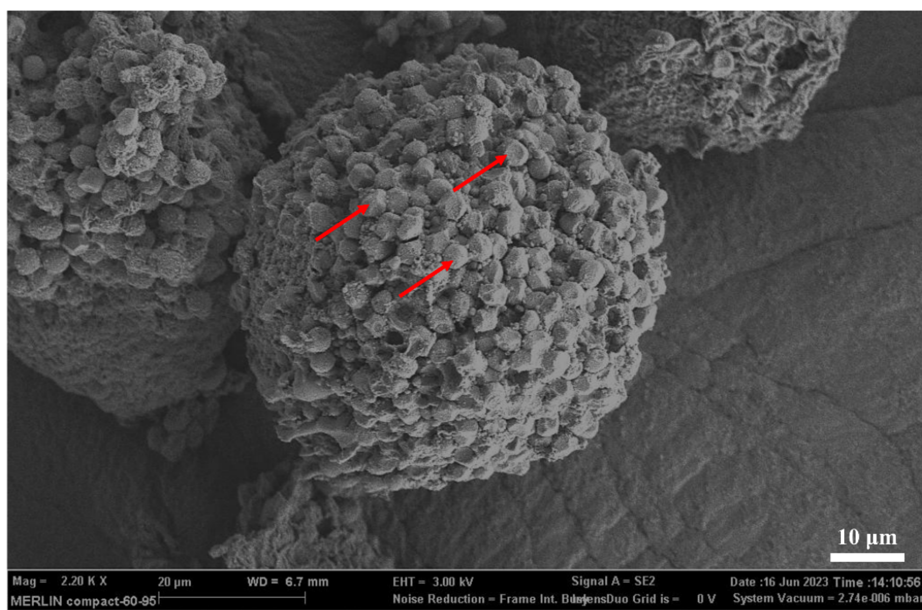

Fig. S4 SEM image of CCM-g-AA(IV) adsorbed red blood cells. Scale bar: 10 µm.

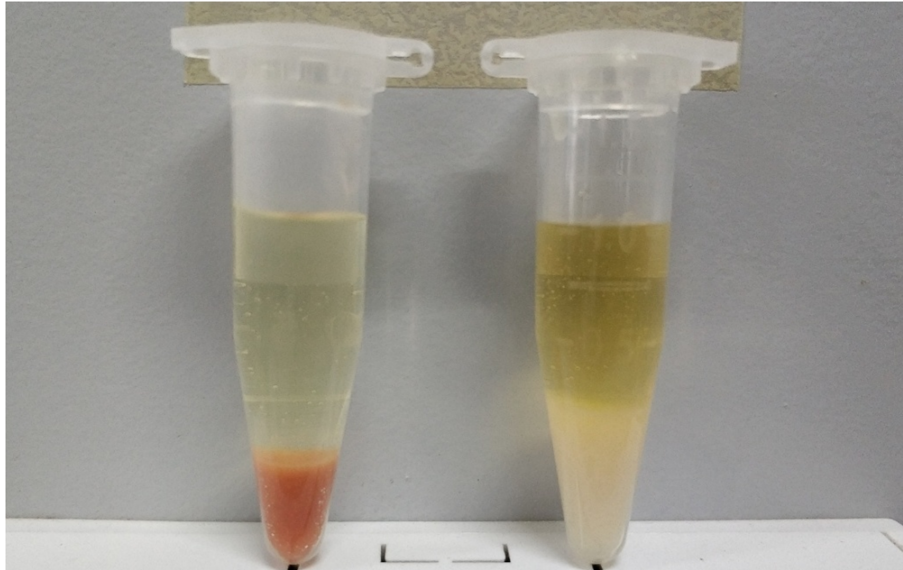

Fig. S5 Adsorption capacity of the microspheres for Fe^{3+} (left: CCM-g-AA(IV), right: CCM).

Fig. S6 In vitro degradation of CCM-g-AA(IV) for 2 days with different concentrations of lysozyme (left: 0.2 mg/mL, right: 2 mg/mL).

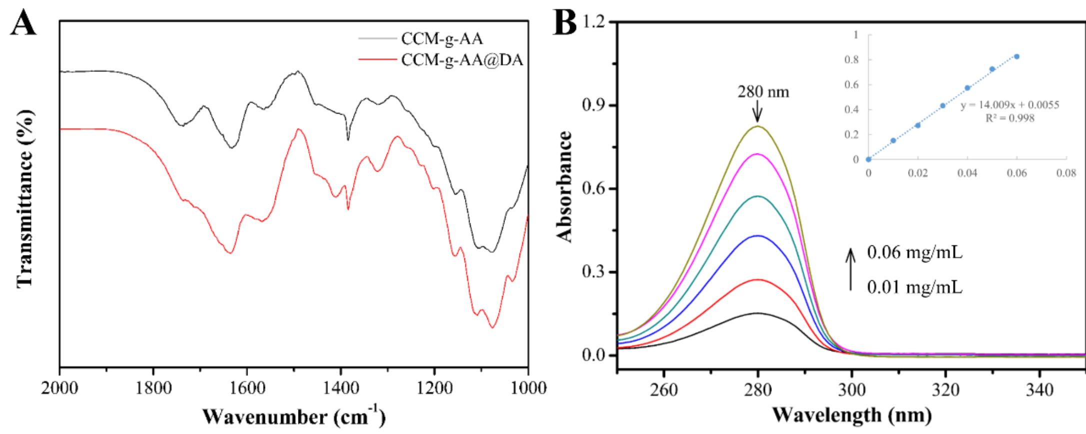

Fig. S7 FTIR of CCM-g-AA@DA microspheres (A) and UV absorption spectra of dopamine (B).

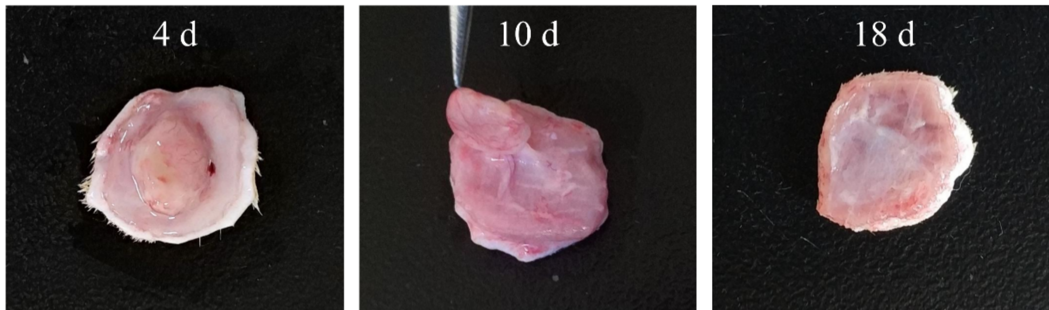

Fig. S8 In vivo rat subcutaneous degradation of the CCM-g-AA@DA microspheres.

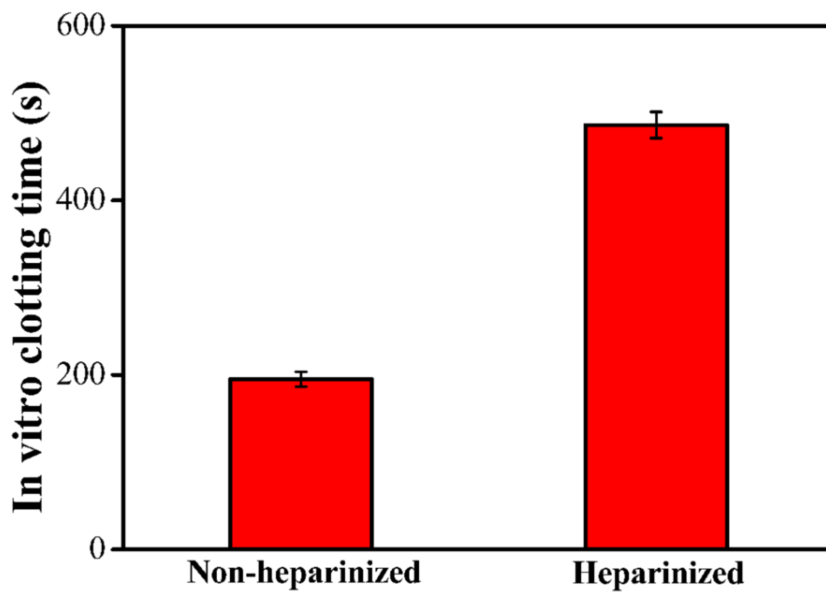

Fig. S9 In vitro clotting time before and after heparin sodium treatment in SD rats. n = 4.