Supporting Information

Investigation of Chitosan and its Derivatives on Red Blood Cells Agglutination

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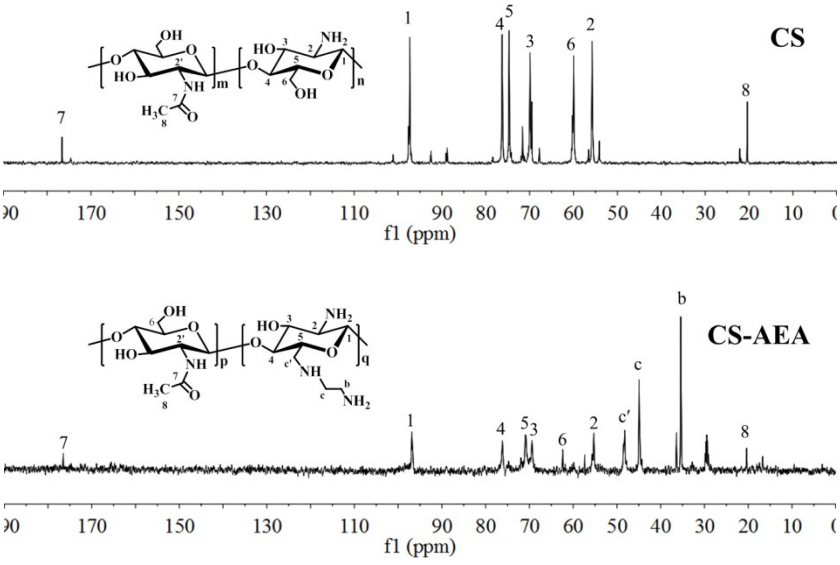
^{13}CNMR spectra of CS, CS-AEA and CS-AEA-AEM were shown in Figure S1. CS exhibits signals at 97.34, 76.27, 74.68, 69.91, 59.97 and 55.75 ppm, which were assigned to C-1, C-4, C-5, C-3, C-6, and C-2 in pyranoid ring respectively. Peaks at 176.58 and 20.41 ppm were assigned to C-7 and C-8 in acetyl group. For CS-AEA, peaks at 35.41 and 36.39 ppm were originated from -CH_2-NH_2, while peaks at 44.94 and 48.21 ppm could be ascribed to signals of -CH_2-NH- marked in the corresponding structural formula. Different from CS-AEA, peaks at 47.62-51.94 ppm represented the existence of different -CH_2- connected with amines in the ^{13}CNMR spectra of CS-AEA-AEM.



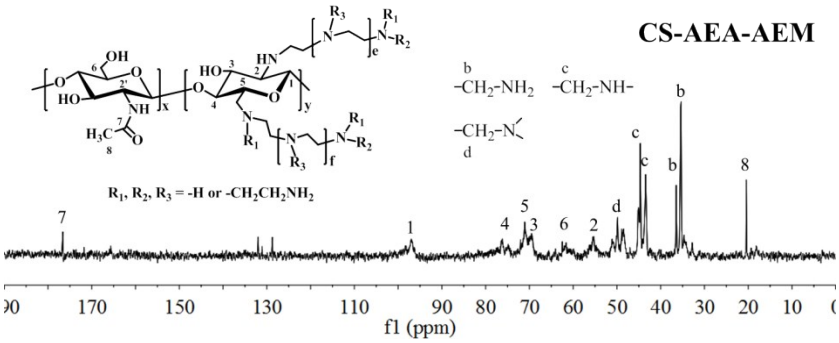


Figure S1. ^{13}CNMR spectra of CS, CS-AEA and CS-AEA-AEM in 1% DCl/D_2O at 25 ^{o}C.

In control samples, RBCs did not agglutinate even after prolonged incubation at the tested pH range (Figure S2). Without CS or its derivatives in solution, changing pH value should have no influence on the agglutination of RBCs.

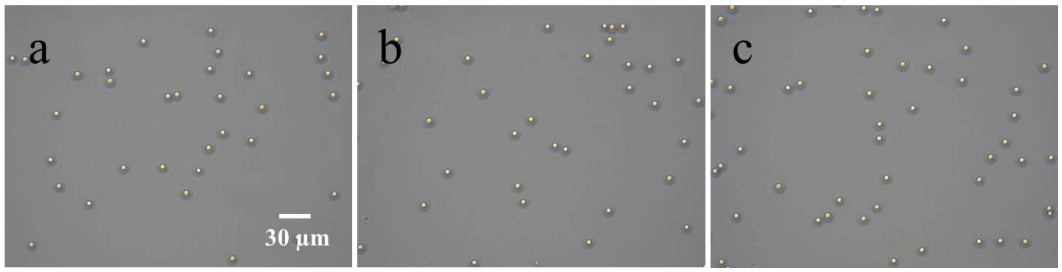


Figure S2. OM images of RBCs agglutination in control samples, a: control-1 at pH 7.4; b: control-2 at pH 7.0; c: control-3 at pH 6.4.

The effect of concentration on RBCs agglutination at pH 7.0 was shown in figure S3. In CS solution, RBCs agglutination was not obvious. No agglutination was found at 0.8 mg/mL (Figure S3a). The agglutination of two RBCs was found occasionally at 2.0 mg/mL (Figure S3d). At 5.0 mg/mL, loose agglutionation of five to ten RBCs was found (Figure S3g). In CS-AEA solution, agglutination was found even at 0.8 mg/mL (Figure S3b). As the concentration increased, agglutination became more obvious (Figure S3e), and tightly large clusters of RBCs were found as the concentration reached 5.0 mg/mL (Figure S3h). In CS-AEA-AEM solution, agglutination was also

found at 0.8 mg/mL (Figure S3c). The increase of agglutination degree was also found with the increasing CS-AEA-AEM concentration (Figure S3f and Figure S3i).

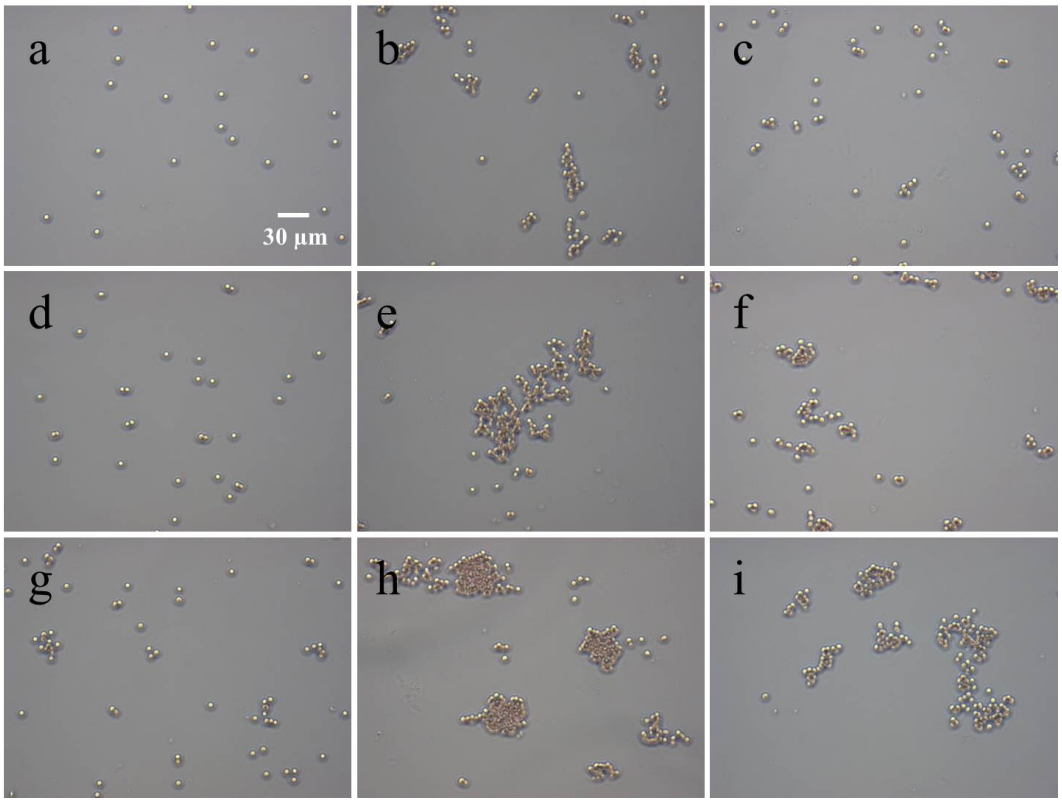


Figure. S3. OM images of RBCs agglutination in CS and its derivates solution at pH 7.0. At 0.8 mg/mL: a. CS, b. CS-AEA, c. CS-AEA-AEM. At 2.0 mg/mL: d. CS, e. CS-AEA, f. CS-AEA-AEM. At 5.0 mg/mL: g. CS, h. CS-AEA, i. CS-AEA-AEM.

The effect of concentration on RBCs agglutination at pH 7.4 was shown in figure S4. In CS solution, RBCs agglutination was not observed at all concentrations (Figure S4a, S4d and S4g). In CS-AEA solution, agglutination was also found even at 0.8 mg/mL (Figure S4b). As the concentration increased, agglutination became more obvious (Figure S4e and S4h) and tight clusters were found at 5.0 mg/mL. In CS-AEA-AEM solution, agglutination of 3-5 RBCs was also found at 0.8 mg/mL (Figure S4c). The increase of agglutination degree was also found with the increasing CS-AEA-AEM concentration (Figure S4f and Figure S4i).

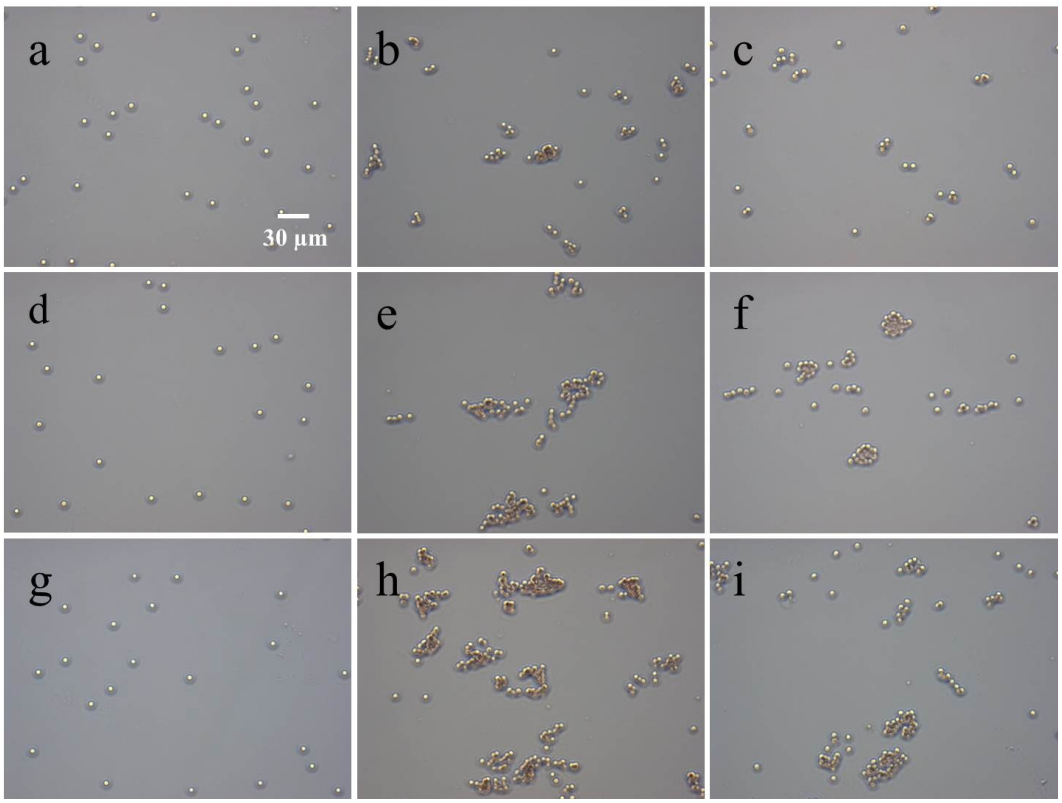


Figure. S4. OM images of RBCs agglutination in CS and its derivates solution at pH 7.4. At 0.8 mg/mL: a. CS, b. CS-AEA, c. CS-AEA-AEM. At 2.0 mg/mL: d. CS, e. CS-AEA, f. CS-AEA-AEM. At 5.0 mg/mL: g. CS, h. CS-AEA, i. CS-AEA-AEM.