Supporting Information

Enhanced osteogenesis of multilayered pore-closed microsphere-immobilizated hydroxyapatite scaffold via sequential delivery of osteogenic growth peptide and BMP-2

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Method

Label reaction of chitosan with fluorescein

On account of chitosan as amino polysaccharide compounds, the amino groups (-NH_2) on the chitosan can be used to react with isothiocyano groups (-N=C=S) on the fluorescein, with the formation of sulfur carbon amino groups, so that the rhodamine B isothiocyanate (RBITC) and fluorescein isothiocyanate (FITC) could be immobilized on the chitosan.

1mg RBITC was dissolved completely in 1mL of anhydrous DMSO. 100 μL of this solution was slowly added dropwise under stirring to the 100 ml of 2.5% chitosan solution achieved by adjusting with 1 mol/L NaOH solution pH to 8.5. The reaction mixture was further stirred for 1 h at 40℃, and then adding 25 μL of ethanol amine so as to terminate the reaction. Free RBITC was removed by dialysis (molecular weight cut off of 1000 Da, MD34MM capacity, Thermo Scientific, USA). The dialyzate was detected until without fluorescence (λex=552nm, λem=588nm). Finally, the resulting wet solid was freeze-dried in vacuo to obtain Rhodamine-B-labeled Cs. The above operations were conducted in the dark.

A certain volume of 2.0 mg/mL FITC methanol solution was added slowly to 10.0 mL of 1% chitosan prepared with 0.1 mol/L acetic acid solution with which 10.0 mL of anhydrous methanol solution was mixed. After the mixture was stirred for 3h at room temperature in the dark, 0.2 mol/L NaOH was poured into the resulting solution to precipitate the crude product. The precipitate was centrifuged at 10000 rpm/min for 15 minutes and washed with methanol aqueous solution (70:30, v/v). Washing and centrifugation for several times, the supernatant fluid was detected until without fluorescence (λex=485nm, λem=535nm). Finally, the resulting labeled product was dissolved in 10.0 mL of 0.1 mol/L acetic acid, dialyzed for 3 d

in the dark and freeze-dried.

Result

Characterization of labeled chitosan

Cs, FITC, RBITC and Cs-FITC, Cs-RBITC samples were measured by infrared spectroscopy. Fig. S1 shows that compared with FITC, the absorption of -OH stretching vibration in the vicinity of the 3440cm^{-1} on Cs-FITC still existed and strength was not weakened, while the absorption peak of N=C=S bond in 2021 cm^{-1} disappeared, showing that the N=C=S involved with -NH_2 addition reaction. Compared with Cs, the location of -NH_2 absorption peak on Cs-FITC between 1645 and 1580 cm^{-1} showed no significant change, indicating that the amino groups in the reaction had little effect on Cs. The results of Cs- RBITC were in agreement with the Cs-FITC.

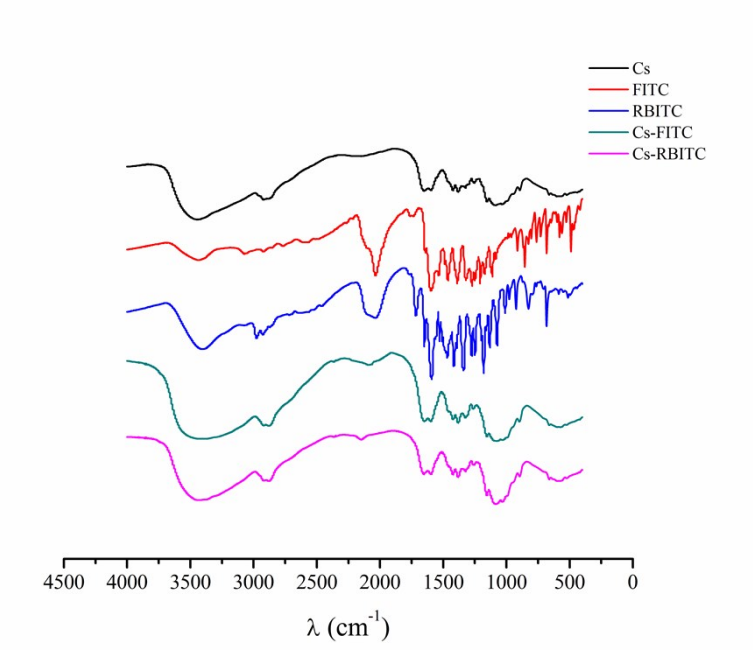

Fig. S1. FT-IR spectrum of Cs, FITC, FITC, Cs-FITC, Cs-RBITC**.**