

Electronic Supplementary Information

**Influence of Molecular Weight and Concentration of Carboxymethyl Chitosan  
on Biomimetic Mineralization of Collagen**

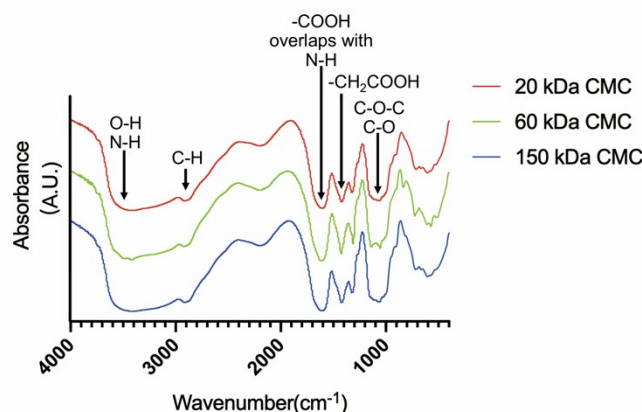
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**Fourier transform infrared spectroscopy (FT-IR) analysis of CMC**

**Methods:** FT-IR analysis of CMC of three different MWs (RuibioC3105, MW 20 kDa, Germany; RuibioC3108, MW 60 kDa, Germany; RuibioC3125, MW 150 kDa, Germany) was performed by an FT-IR spectrometer (Nicolet 6700-Contiium, Thermo Scientific, Waltham, MA, USA). Each spectrum was the result of signal-averaging of 32 scans at a resolution of 2 cm<sup>-1</sup> and the wavenumber ranged from 400 to 4000 cm<sup>-1</sup>.

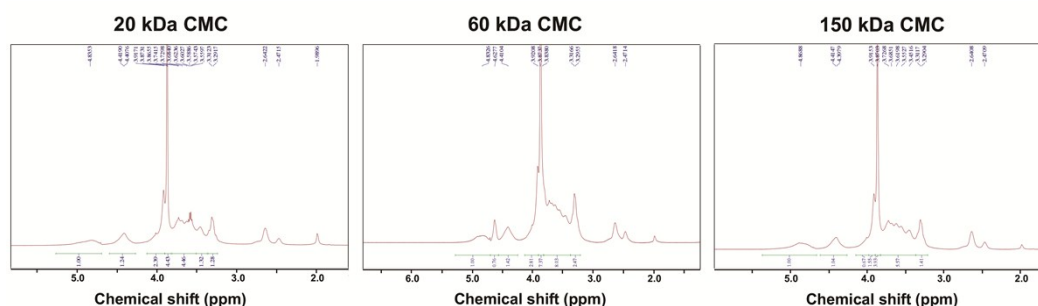


**Fig. S1** FT-IR spectra of CMC of three different MWs. Basic characteristic peaks at 1597-1650 cm<sup>-1</sup> (-COOH group overlaps with N-H bend), 1414-1401 cm<sup>-1</sup> (-CH<sub>2</sub>COOH group), 3455-3445 cm<sup>-1</sup> (O-H and N-H stretch), 2923-2867 cm<sup>-1</sup> (C-H stretch) and 1154-1029 cm<sup>-1</sup> (C-O and C-O-C stretch) are labelled<sup>1,2</sup>.

## <sup>1</sup>H-Nuclear magnetic resonance (NMR) analysis of CMC

**Methods:** Proton spectra of CMC of three different MWs were obtained using an NMR spectrometer (Bruker Avance III, Bruker BioSpin, Switzerland) operating at a frequency of 500 MHz under the normal conditions. Samples were prepared by dissolving in deuterium oxide (D<sub>2</sub>O). Chemical shifts were referenced using tetramethylsilane as internal standard. Degrees of substitution (DS) of different carboxymethyl groups were determined based on the <sup>1</sup>H NMR signal areas<sup>3, 4</sup>.

**Results:** The proton NMR spectra of CMC of three different MWs showed chemical shifts at 3.2-3.5 ppm and 4.2-4.5 ppm (Fig. S2), which could be assigned to monocarboxymethyl substitution on amino and hydroxyl groups respectively<sup>1, 3</sup>. The resonance signals of the protons from N, N-dicarboxymethyl groups could be found at around 3.9 ppm<sup>1, 5</sup>, which were larger than those of monocarboxymethylation in all the spectra. DS of N, N-dicarboxymethyl groups of CMC of three different MWs were close to 100%, while DS of O-carboxymethyl groups were approximately 62%, 40% and 52% respectively. DS of N-carboxymethyl groups were not determined here due to small signals overlapping with those of protons from chitosan.



**Fig. S2** Proton NMR spectra of CMC of three different MWs.

## References

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