Facile preparation of Ca(II) carboxymethyl cellulose complex with enhanced calcium bioavailability for treatment of osteoporosis

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**SUPPLEMENTARY EXPERIMENTS**

**Calcium solubility Test.** 10 µg/mL of CaCMC and CaCl$_2$ were separately dissolved in deionized water, the solutions pH was adjusted to 2.0~8.0. After 2 h incubation at 37 °C, the solutions were centrifuged at 10000g for 10 min. The total calcium amount in the supernatant was determined by ICP.

20 mg of CaCl$_2$ or CaCMC was dissolved in 50 mL distilled water. The pH was adjusted to 2.0 with 1N HCl. 0.5 mg pepsin-HCl digestion was added, the mixture was incubated in a water bath for 2 h at 37°C. After the gastric digestion simulation model, the pH of the solution was regulated to 7.4 by 0.5 M NaOH. Subsequent, a pancreatin-bile salt mixture was added, and incubated for another 2 h. The reaction was stopped by an ice bath for 15 min. Proteases were inhibited by a boiling water bath for 4 min and then cooled in an ice bath. The digested solution was centrifuged at 13500 rpm for 30 min at 4°C. The supernatants were collected for next use.

**Cell culture.** NIH3T3 and HeLa cells were cultured in DMEM with 10 % (v/v) fetal bovine serum (FBS, Invitrogen) in 5 % CO$_2$ at 37 °C. The CCK-8 assay was performed for *in vitro* cytotoxicity of CaCMC, The CaCMC was suspended with PBS. Subsequently, the NIH3T3 and HeLa cell suspension was added. The final CMC concentration was 50 µg/mL. The mixed suspension was seeded into a 96-well flask with $5 \times 10^3$ cells per well and incubated for 48 h. Thereafter, 10 µL CCK-8 per well was injected and incubated for 2 h at 37 °C under 5% CO$_2$. The optical density was measured at 450 nm using a microplate reader (Wallac 1420 Victor).

**Measurements.** FT-IR spectra were achieved by an infrared spectrometer (Nicolet FT-170SX). Morphology of nanocellulose hydrogel was performed by Field-Emission Scanning Electron Microscope (FE-SEM, FEI, Sirion 200). Emission spectra were noted by a Shimadzu RF-5301 spectrophotometer. X-ray photoelectron spectroscopy (XPS) was investigated on a PHI-5702 multifunctional spectrometer using Al Kα radiation. $^1$H-NMR spectrums were collected on a JEOL ESC 400M instrument. The mechanical properties of the nanocellulose hydrogels were described by a rheometer (AR-1000 TA Instruments). The
mechanical properties of the nanocellulose hydrogels were characterized by a rheometer with a cone and plate geometry (AR-1000 TA Instruments).

**Protocol of UV-vis absorption.** The UV-vis absorption is spectrophotometry is a physicochemical analysis technique, the principle is based on the light-absorption capacity of a product where light of wavelength $\lambda$ through a medium absorbing a distance $l$ with incident intensity $I_0$, and it emerges with an intensity of $I$. The absorbance at the wavelength solution is expressed in terms of $I$ and $I_0$ is given by the Beer-Lambert law:

$$A = \log_{10} \left( \frac{I_0}{I} \right) = \varepsilon l c$$

$c$ is the molar concentration of the absorbing species, $\varepsilon$ is the molar extinction coefficient of this species to the wavelength $\lambda$, and $l$ is the length traveled by the light in the absorbing medium.

According to the data of Fig. S2, $\varepsilon$ is 550 L. cm$^{-1}$. mol$^{-1}$ for DPY. The absorbance of CMC-DPY is 0.016, so calculated as below:

$$C_{\text{CMC-DPY}} = \frac{A}{\varepsilon} = 2.9 \times 10^{-2} \text{ mol L}^{-1}$$

Here we used 1 mL (2 wt%) CMC-DPY for the test, so

$$C_{\text{CMC}} = \frac{0.002}{25,000} \times 10^{-3} = 8 \times 10^{-5} \text{ mol L}^{-1}$$

THE molar ratio $N = \frac{C_{\text{CMC-DPY}} V}{C_{\text{CMC}} V} = 362.5$

This means each CMC can bind about 362 DPY molecular.
SUPPLEMENTARY FIGURES AND TABLES

Fig. S1. $^1$H NMR spectra of CMC, DPY, CMC-DPY and CaCMC in DMSO-$d_6$. 
Fig. S2. Plot of the absorbance against the concentration of DPY.

Fig. S3. XPS spectra of (1), (2), (3) and (4).
**Fig. S4.** High-resolution of C1s and O1s XPS spectra of CMC (A, B), and C1s, O1s and N1s XPS spectra of CMC-DPY (2) (C-E).

Fig. S4 shown the C1s spectrum of CMC and CMC-DPY have the same peaks at 284.7, 286.6, and 288.8 eV assigning to C-C/C=C, C-O-C, and O-C=O species, which belong to pyran ring, and carboxyl species. Except for CMC-DPY, the decomposed peak at 288.6 eV belongs to O=C-N in C1s spectrum, the decomposed peak at 531.08 eV belongs to O=C-N in O1s spectrum, and the decomposed peak at 401.6 eV belongs to O=C-N in N1s spectrum, proving the formation of amide bond.
As shown in Fig. S5, the C1s spectrum of CMC composite can be decomposed into three main peaks at 284.7, 286.6, and 288.8 eV assigning to C-C/C=, C-O-C, and O-C=O species, which belong to pyran ring, and carboxyl species. For (3), the decomposed peak at 288.5 eV belongs to O=C-N in C1s spectrum (Figure S4A), the decomposed peak at 530.8 eV belongs to O=C-N in O1s spectrum (Figure S4B), and the decomposed peak at 400.6 eV belongs to O=C-N in N1s spectrum (Figure S4C), which proving the formation of amide bond. For CaCMC, the decomposed peak of O=C-N at 288.3 eV in C1s spectrum, 530.9 eV in O1s spectrum and 401.9 eV in N1s spectrum. Since the coordination of Ca$^{2+}$ with O and N, this caused the upshift of the O1s and N1s spectrum for CaCMC.
Fig. S6. Variation of storage modulus, $G'$, and loss modulus, $G''$, in strain-sweep (A) and frequency-sweep (B) experiments of CaCMC hydrogel at 2 wt%.

Fig. S7. (A) SEM images of CMC-DPY, (B) Dark field of STEM and element mapping images of CMC-DPY.
Fig. S8. Cytotoxic assay of CaCMC and CMC-DPY in MG63 and Saos2 cells.
Fig. S9. Metrology index of (A) body weight, (B) length of the femur, and (C) femur weight.
Fig. S10. ALP activity in the serum.

Fig. S11. The content of (A) ALT and (B) AST in serum.

<table>
<thead>
<tr>
<th>Group</th>
<th>BMD (g/cm³)</th>
<th>BV/TV (%)</th>
<th>Tb.Th (mm)</th>
<th>Tb.N (1/mm)</th>
<th>Tb.Sp (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.071</td>
<td>4.98</td>
<td>0.125</td>
<td>0.373</td>
<td>0.780</td>
</tr>
<tr>
<td>Model</td>
<td>0.057 ↓</td>
<td>3.77 ↓</td>
<td>0.108 ↓</td>
<td>0.337 ↓</td>
<td>0.964 ↑</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>0.064</td>
<td>4.64</td>
<td>0.119</td>
<td>0.354</td>
<td>0.907</td>
</tr>
<tr>
<td>Low-dose</td>
<td>0.068</td>
<td>4.68</td>
<td>0.116</td>
<td>0.344</td>
<td>0.737</td>
</tr>
<tr>
<td>High-dose</td>
<td>0.075 ↑</td>
<td>8.36 ↑</td>
<td>0.135 ↑</td>
<td>0.691 ↑</td>
<td>0.446 ↓</td>
</tr>
</tbody>
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