Supporting Information

ZIF-8 Modified Multifunctional Bone-Adhesive Hydrogels Promoting Angiogenesis and Osteogenesis for Bone Regeneration

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Methods

Fabrication of CA-CS

According to the established method, standard EDC chemistry was used to conjugated catechol groups onto the chitosan backbone[1]. In brief, the solution of chitosan and Hydrocaffeic acid were reacted under the catalysis of EDC for 12h. Then, the product was dialyzed (MWCO: 12000-14000, SpectraPor, USA) in acidified DI H_2O (pH 5.0, HCl) for 2 days, and in DI H_2O for 4 h. After lyophilization , the final product (CA-CS) could be obtained. The synthesis of CA-CS was confirmed by using either ^1H NMR (Bruker Avance, 500 MHz) or UVvis spectrophotometer (UV3600, Shimadzu, Japan).

Fabrication of ZIF-8 NPs

MeIm (2.27 g) and zinc nitrate hexahydrate (0.11 g) were dissolved in DI H_2O (40ml), and stirred gently for 20 min. The obtained solution was then transferred to Teflonlined autoclaves which was heated at 37 °C for 6 h. The ZIF-8 nanoparticles（ZIF-8 NPs）were obtained by thoroughly rinsing with methanol (10 mL × 3) and drying at 37 °C for 24 h [2]. The morphology of the ZIF-8 NPs was analyzed with the scanning electron microscope (SEM) (KYKY Technology Development Ltd.). A X' Pert pro MPD diffractometer (Philips, Japan) and a Thermo Fisher Scientific FT-IR spectrometer (Nicolet 6700, USA) were measured to analyze characteristics of ZIF-8 NPs.

Results

Characterization of CA-CS

CA-CS was synthesized via EDC chemistry by forming an amide bond between primary amine groups in chitosan and a carboxylic acid group in hydrocaffeic acid. The success of this reaction was quantified by 1H-NMR measurement (Figure S1) that determined the degree of catechol conjugation (DOC) onto the chitosan backbone [1]. Specifically, the DOC value of 13.9% we got was calculated by dividing the integration value of the catechol proton peaks which appeared from 6.5 to 6.7 ppm by another value of the acetyl group protons appeared from 1.88 to 1.95 ppm multiplied by 5 (the manufacturer provides us 20% acetylation of chitosan). In addition, the absorbance at 280 nm of UV-vis spectroscopy caused by hydrocaffeic acid conjugation also confirmed successful synthesis of CA-CS (Figure S2).

Characterization of ZIF-8 NPs

We examined the morphology of the ZIF-8 NPs using SEM, and then used the ImageJ software to analyze the obtained micrograph (Figure S3). The ZIF-8 NPs prepared by hydrothermal method were rhombic dodecahedral in shape and approximately 280 nm in size. To investigate the phase purity of ZIF-8 NPs in hydrogels, we collected ZIF-8 NPs in the mother liquor and analyzed them using spectroscopy FT-IR and XRD. The XRD pattern (Figure S4) of the prepared ZIF-8 NPs were in good agreement with the simulated crystal image. As shown in the FT-IR spectrum (Figure S5), the position of the peak and its distribution were consistent with those reported in the literature [3]. Both of the XRD pattern and FT-IR spectrum indicated that the crystals were pure phase. Therefore, the nanofillers prepared in our hydrogel proved be to ZIF-8 NPs without any other

impurities.

Figures

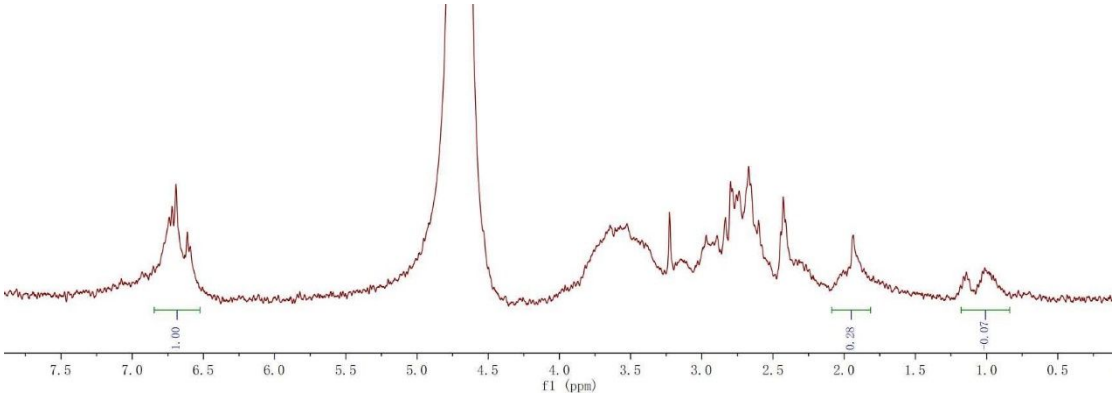

Figure S1 1H-NMR spectrum of the synthetic CA-CS.

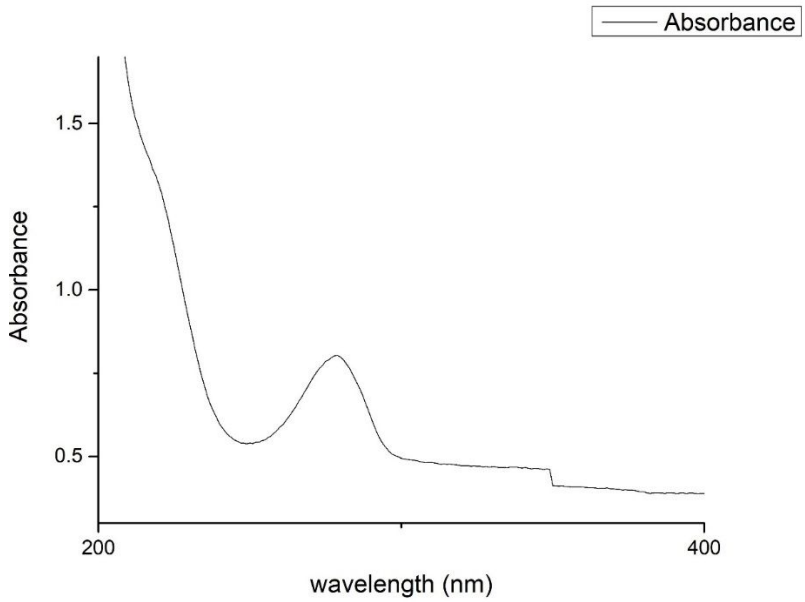

Figure S2 UV-Vis spectroscopy of the synthetic CA-CS.

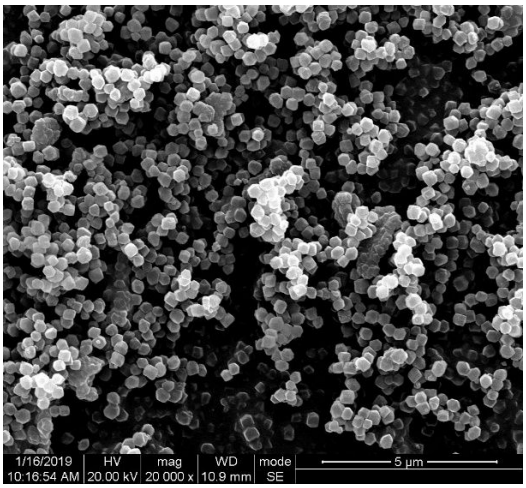

Figure S3 SEM images of nanoscale ZIF-8.

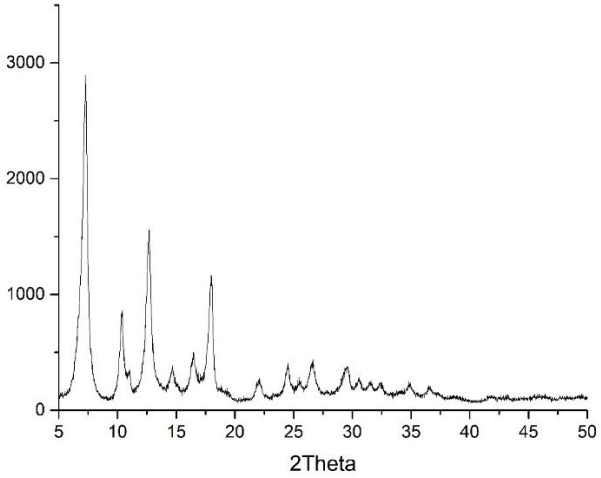

Figure S4 XRD patterns of the as-prepared ZIF-8 crystals

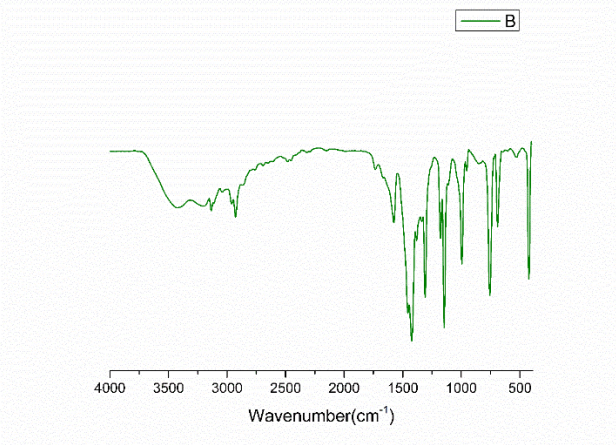

Figure S5 FT-IR spectra of as-prepared ZIF-8 crystals

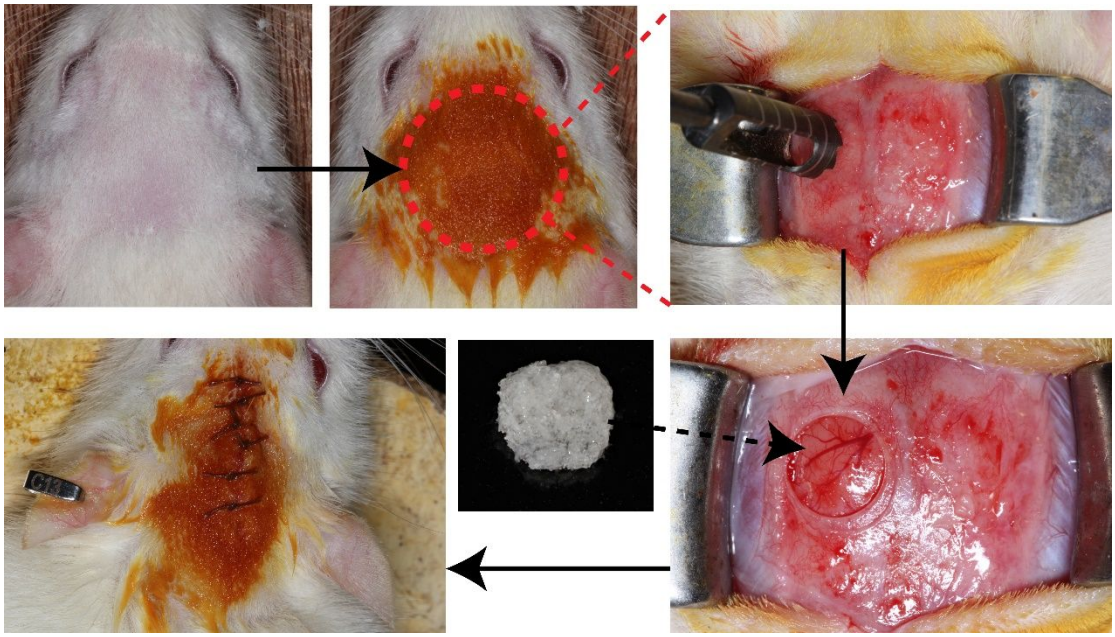

Figure S6 The surgery procedure to perform a cranial defect model.

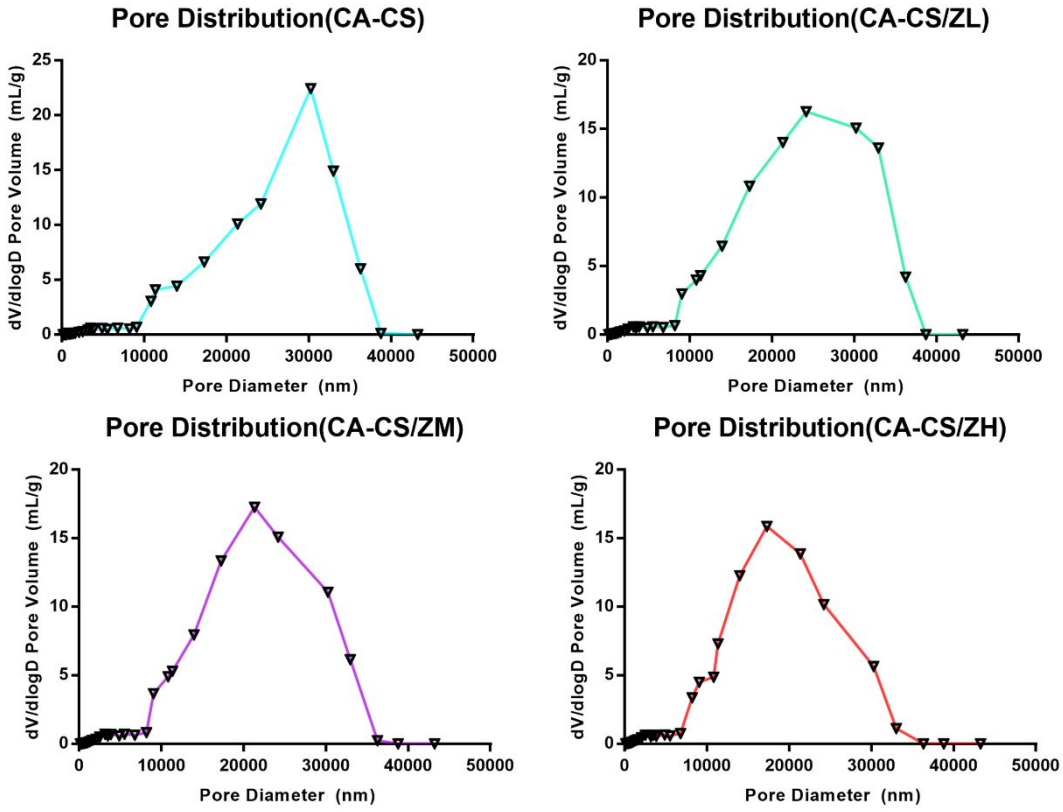

Figure S7 The pore size distribution of CA-CS, CA-CS/ZL, CA-CS/ZM, CA-CS/ZH.

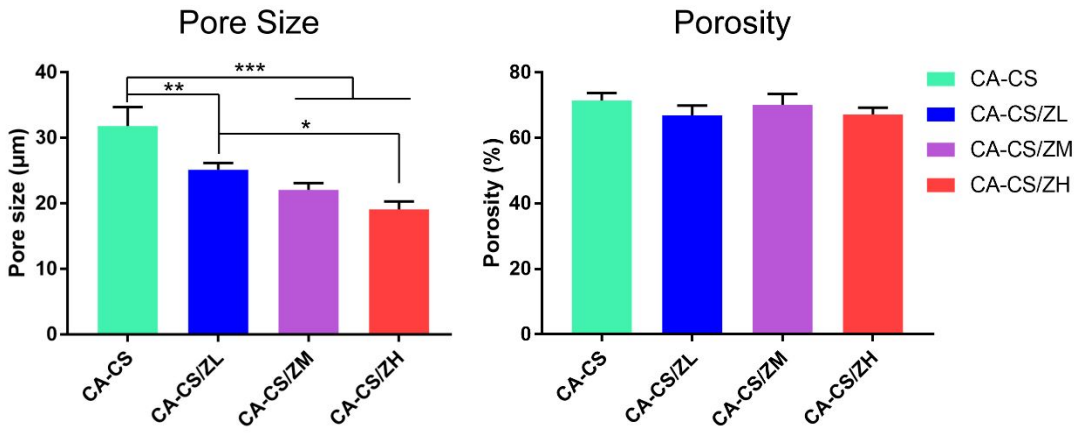

Figure S8 The pore size and porosity CA-CS, CA-CS/ZL, CA-CS/ZM, CA-CS/ZH.

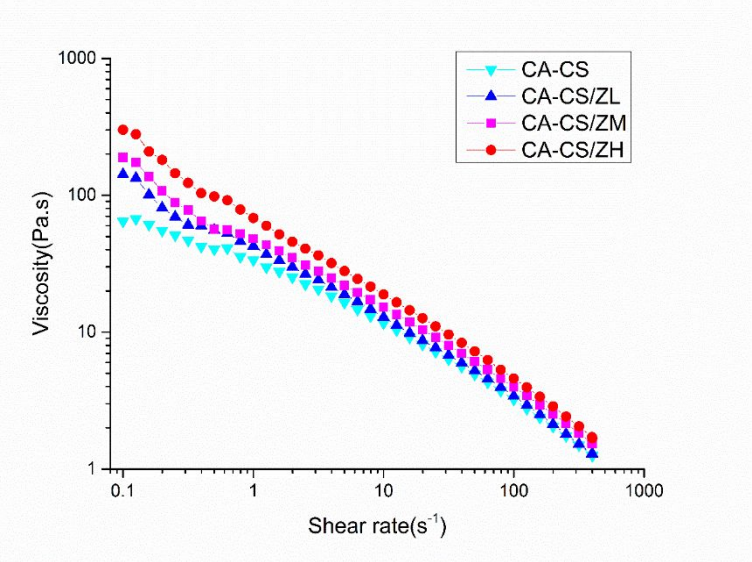

Figure S9 Relationships between viscosity and shear rate for different samples at 28 °C.

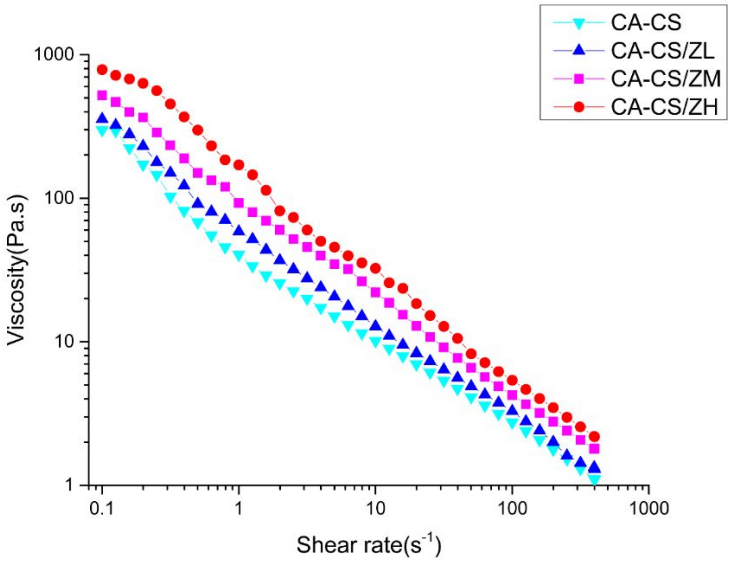

Figure S10 Relationships between viscosity and shear rate for different samples at 37 °C.

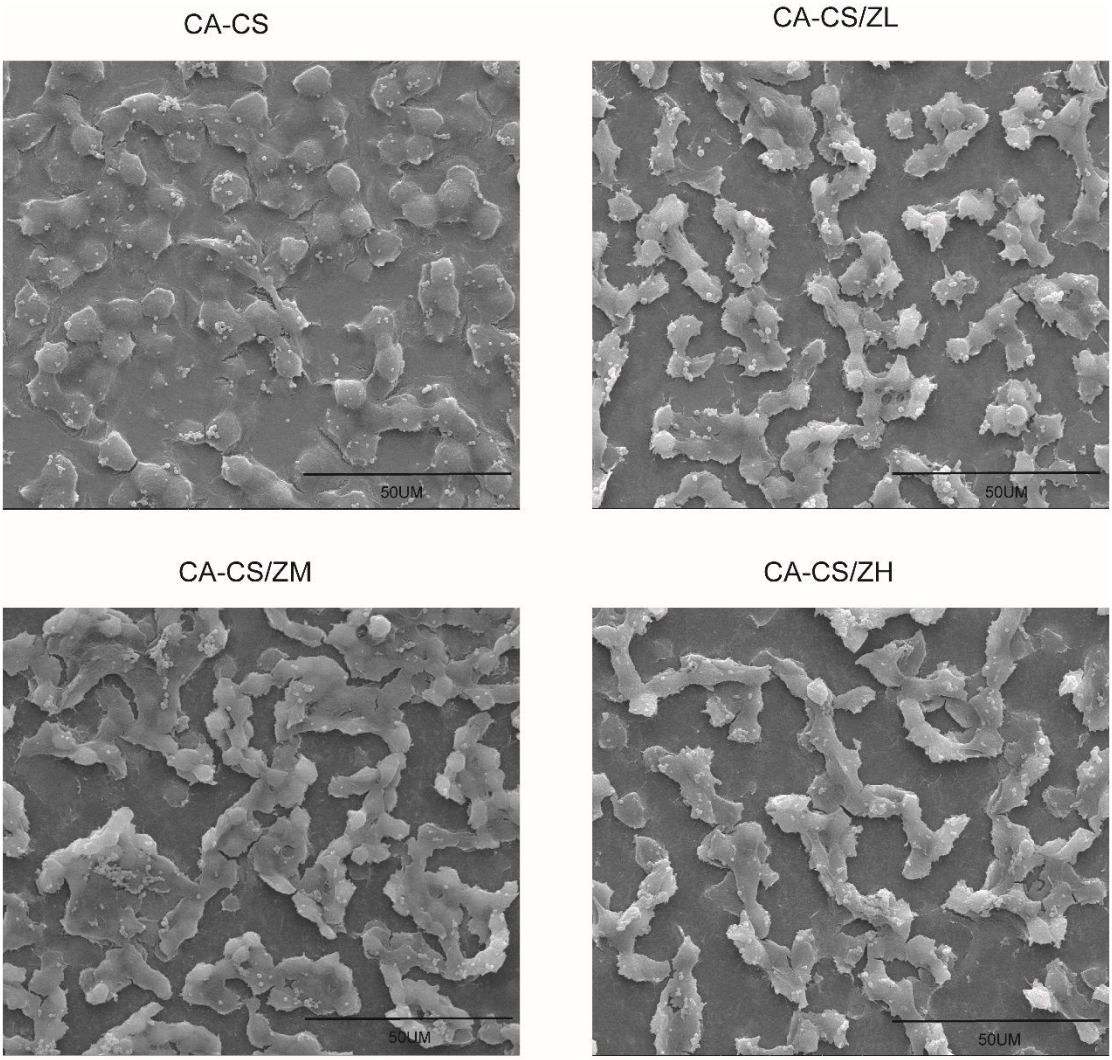

Figure S11 SEM morphology of rBMSCs cultured on the hydrogel's surfaces for 6 h.

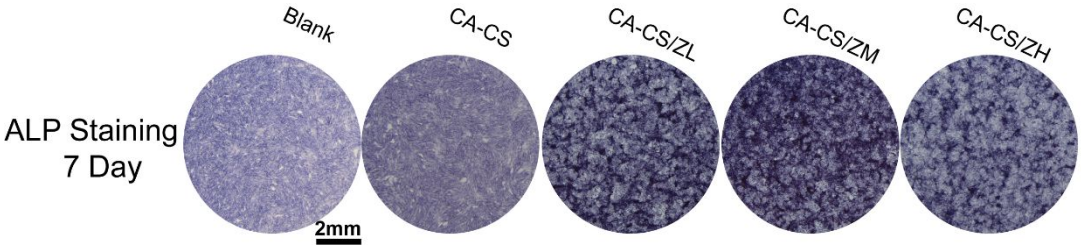

Figure S12 Alkaline phosphatase secretion of rBMSCs cocultured with different hydrogels for 7 days.

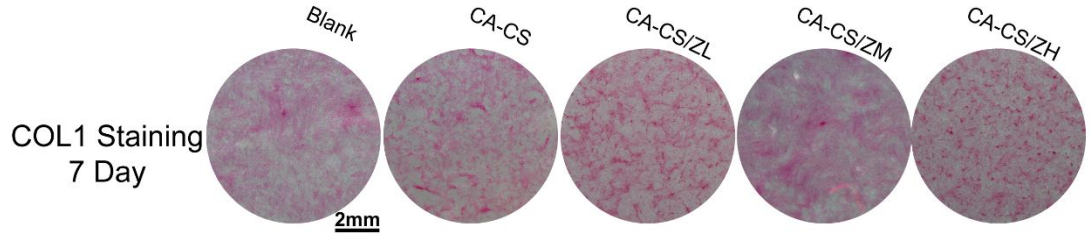

Figure S13 Collagen 1 secretion of rBMSCs cocultured with different hydrogels for 7 days.

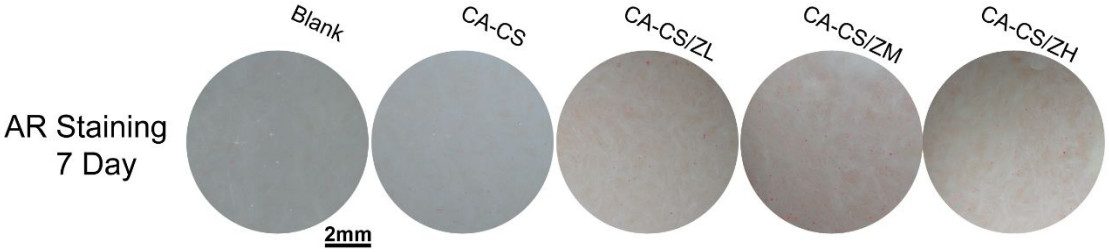

Figure S14 Matrix mineralization of rBMSCs cocultured with different hydrogels for 7 days.

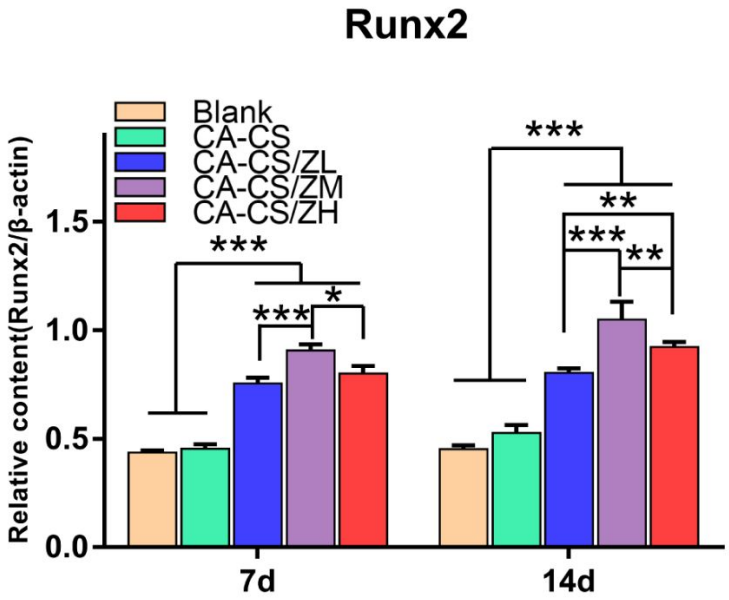

Figure S15 Quantitative result of Runx2's Western-blot in rBMSCs analysis for CA-CS and CA-CS/Z groups.

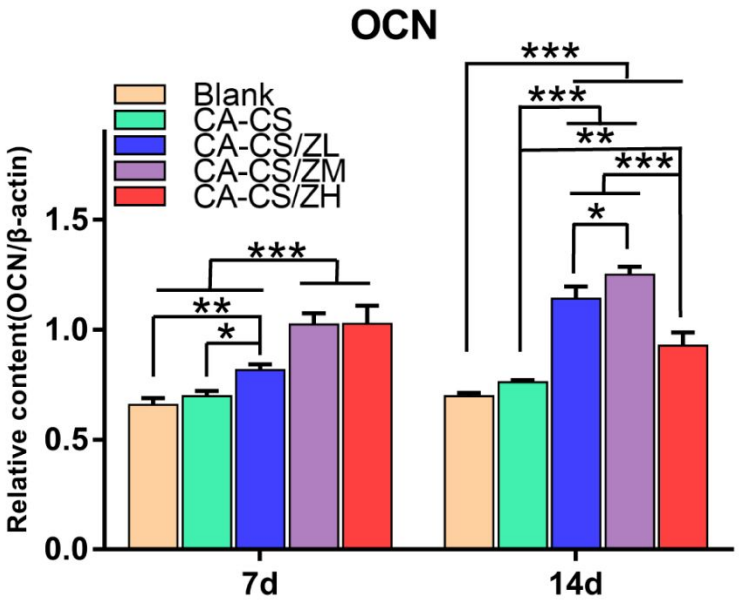

Figure S16 Quantitative result of OCN's Western-blot in rBMSCs analysis for CA-CS and CA-CS/Z groups.

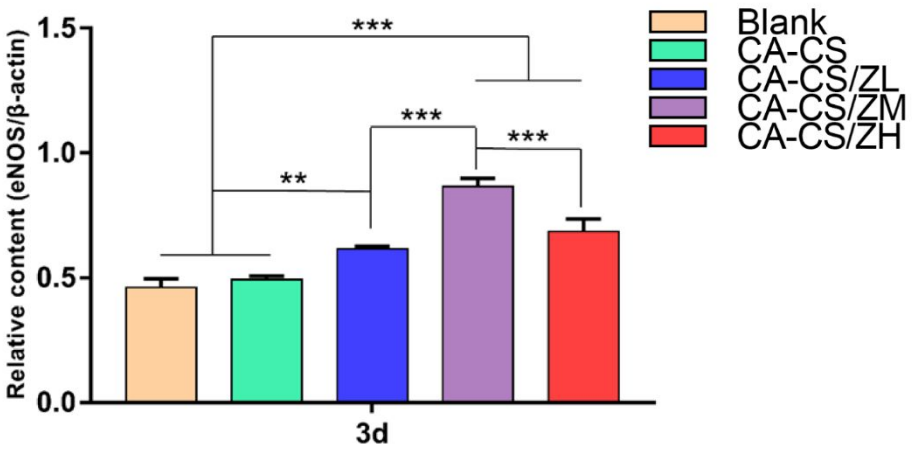

Figure S17 Quantitative result of eNOS's in HUVECs Western-blot analysis for CA-CS and CA-CS/Z groups.

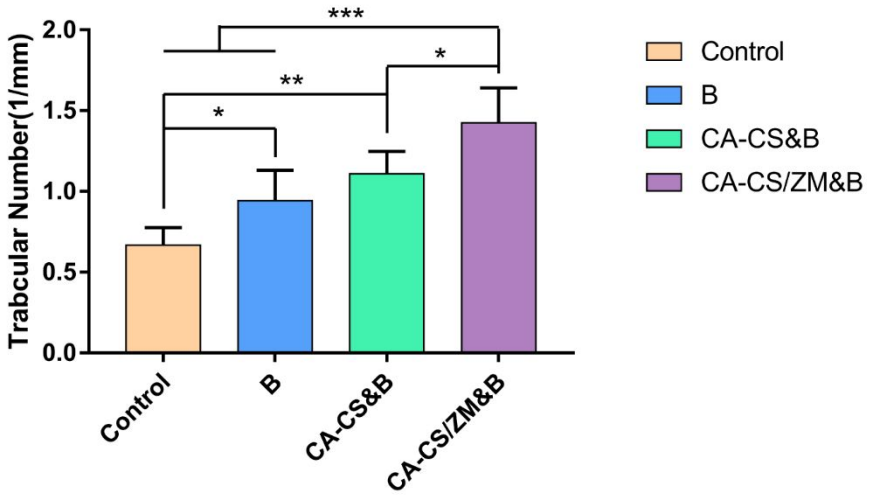

Figure S18 The nubmer of trabcluar accroding to Micro-CT.

Reference

[1] Ryu JH, Lee Y, Kong WH, Kim TG, Park TG, Lee H. Catechol-functionalized chitosan/pluronic hydrogels for tissue adhesives and hemostatic materials. Biomacromolecules. 2011;12:2653-2659.

[2] Zhang X, Chen J, Pei X, Wang J, Wan Q, Jiang S, et al. Enhanced Osseointegration of Porous Titanium Modified with Zeolitic Imidazolate Framework-8. ACS Appl Mater Interfaces. 2017;9:25171-25183.

[3] Venna SR, Carreon MA. Highly Permeable Zeolite Imidazolate Framework-8 Membranes for CO2/CH4 Separation. Journal of the American Chemical Society. 2010;132:76-+.