Biomimetic and Cell-mediated Mineralization of Hydroxyapatite by Carrageenan Functionalized Graphene Oxide

Hongyan Liu[ab], Ju Cheng[c], Fengjuan Chen[ab], Fengping Hou[a], Decheng Bai[c], Pinxian Xi[ab], Zhengzhi Zeng*[ab]*

a Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province, State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China. E-mail: xipx@lzu.edu.cn, zengzhzh@lzu.edu.cn.

b The Research Center of Biomedical Nanotechnology, Lanzhou University, Lanzhou 730000, P. R. China.

c Key Lab of Preclinical Study for New Drugs of Gansu Province, School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, PR China

Experimental section

Quantify of ALP activity

At each culturing period, the cell-grown substrates were rinsed with PBS, and incubated in 500 μL Tris buffer (10 mM, pH 7.5) containing 1% Triton X-100 for 30 min. Next, 100 μL of the lysate was added to a 96-well tissue culture plate containing 100 μL of *p*-NPP solution. The absorbance of the solution at 405 nm was measured by a microplate reader (Powerwave X, Bio-Tek USA). The ALP activity was determined by the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (*p*-NPP).

Alizarin red staining

Calcium mineralization was determined by alizarin red S staining of MC3T3-E1 cells at 14 days of culture. This was based on Alizarin red-S (ARS) staining dye, which binds selectively calcium salts. The substrates with cells were washed three times with PBS and fixed with 4% formaldehyde for 1 h, washed five times carefully with distilled H_2O and then stained with ARS (50 mM) for 30 min at room temperature. After several washes with distilled H_2O to remove excess dye, substrates were examined under the optical microscope.

Supplementary spectra

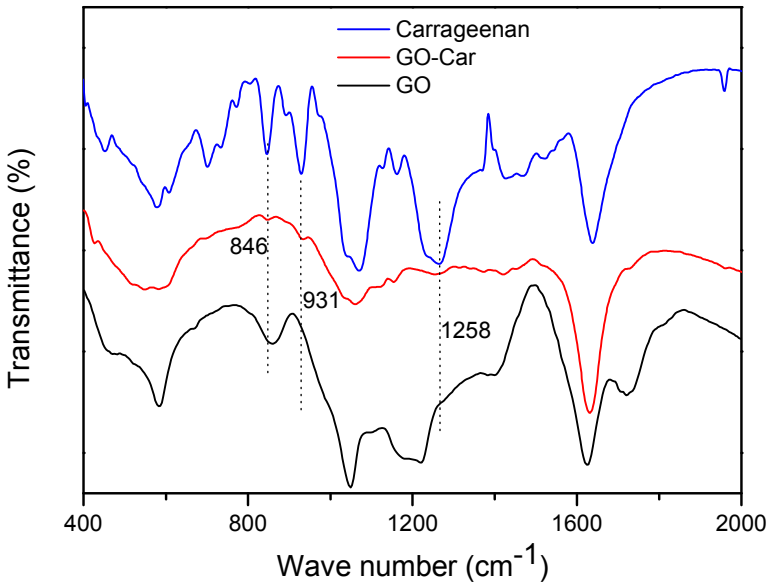

Figure S1 FTIR spectra of GO, carrageenan, and GO-Car composite.

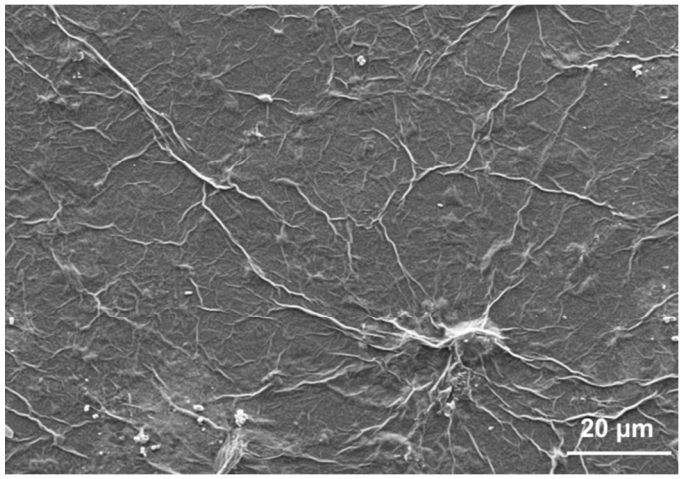

Figure S2 SEM images of GO after mineralization.

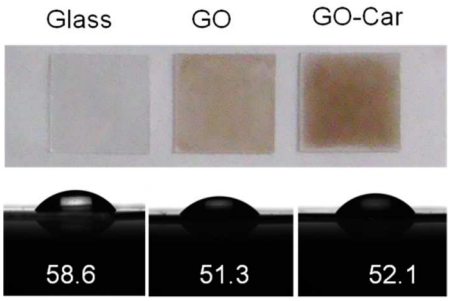

Figure S3 Optical images of substrates modified with GO-based nanomaterials (top) and water contact angles of glass, GO, and GO-Car substrates.

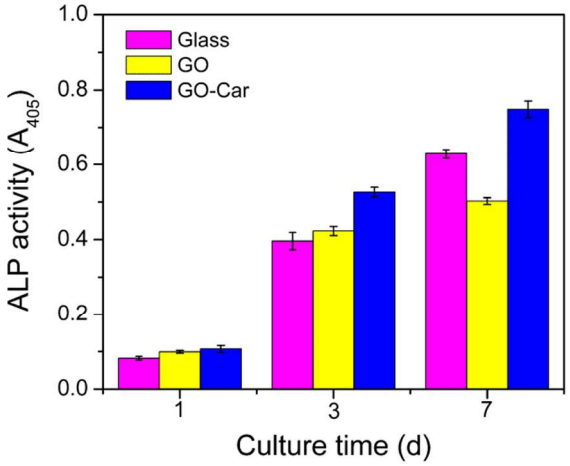

Figure S4. Quantitative analysis of the ALP activity of MC3T3-E1 cells cultured on glass, GO, and GO-Car for 1, 3, and 7 days.

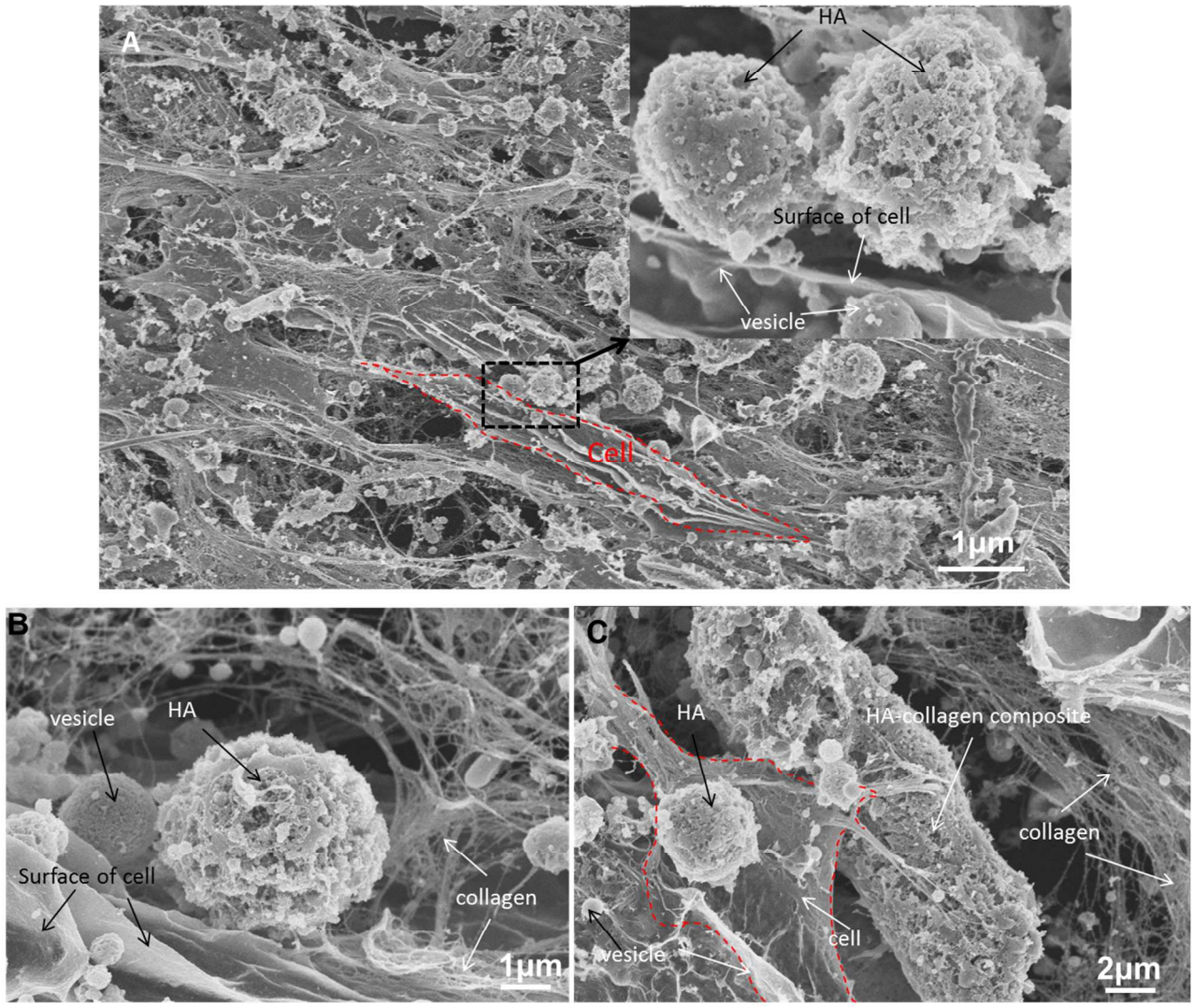

Figure S5 Typical SEM images of MC3T3-E1 cells cultured on GO-Car (A, C) and GO (B) for 14 days showing cells, HA, collagen, and vesicles.

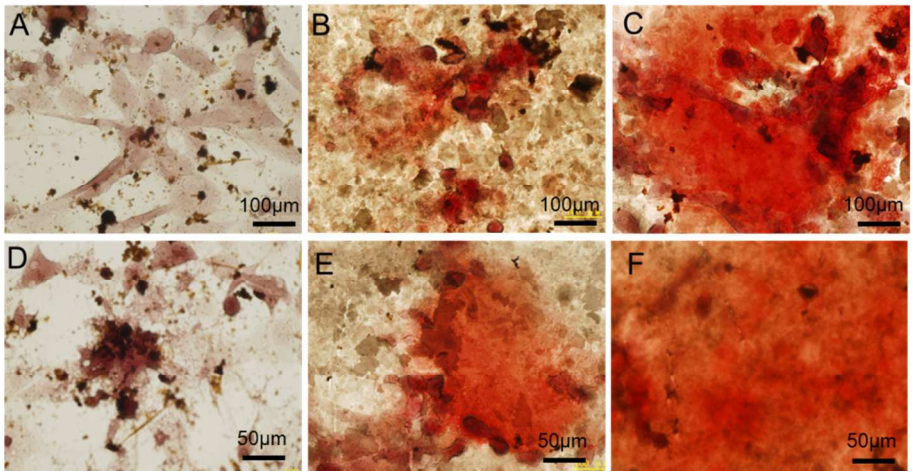

Figure S6 Alizarin Red S staining of mineral on glass (A, D), GO (B, E), and GO-Car (C, F) after 14 days mineralization