Supporting information for:

Development of CaCO₃ microspheres based composite hydrogel for dual delivery of growth factor and Ca to enhance bone regeneration

Yihong Gong, a,b Yiling Zhang, a,b Zhinan Cao, a,b Feng Ye, c Zefeng Lin, d,e and Yan Li a,b*

a Guangdong Provincial Key Laboratory of Sensor Technology and Biomedical Instrument, School of Biomedical Engineering, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
b Guangdong Provincial Engineering and Technology Center of Advanced and Portable Medical Devices, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
c Department of Pharmacology, Beijing Anzhen Hospital, Capital Medical University, Beijing Institute of Heart, Lung and Blood Vessel Disease, Beijing, P.R. China
d Department of Orthopedics, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, 510010, China.
e Guangdong Key Laboratory of Orthopedic Technology and Implant Materials, Guangzhou, 510010, China.

*Corresponding author: Yan Li, Telephone: +86-20-39332146, Fax: +86-20-39332146, Email: liyan99@mail.sysu.edu.cn
Figure S1. Digital images of tibia bone tissue with created defects after implanting CaCO$_3$/fibrin-gel composite hydrogels for (A) 30 days and (B) 60 days. FC-B was CaCO$_3$/fibrin-gel composite hydrogel with BMP-2 and FC was CaCO$_3$/fibrin-gel composite hydrogel without BMP-2.

Figure S2. Cumulative release of (A) Ca$^{2+}$ and (B) CaCO$_3$ from casein and heparin co-functionalized CaCO$_3$ microspheres. After sterilization (soaking in 75% ethanol and then UV radiation), 16 mg CaCO$_3$ microspheres were suspended in 3 ml PBS solution containing 1% (v/v) penicillin/streptomycin and then incubated in 37°C water bath. At predetermined time points, 1.5 ml solution was taken out and replenished with fresh PBS. Ca$^{2+}$ concentration was quantified using a Calcium-O-Cresolphthalein complexone method and the released amount of CaCO$_3$ was calculated accordingly by assuming that only Ca$^{2+}$ and CO$_3^{2-}$ ions were released from microspheres. Three replicates were prepared. Data = mean ± SD.