Supporting information for

Fabrication of Uniform Casein/$CaCO_3$ Vaterite Microspheres and Investigation on Its Formation Mechanism

Yan Li,[a,b] Ximei Li,[a,b] Zhinan Cao,[a,b] Yue Xu,[c,d] Yihong Gong,[a,b*] and Xuetao Shi[e]

[a]Guangdong Provincial Key Laboratory of Sensor Technology and Biomedical Instrument, Department of Biomedical Engineering, School of 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 Orthodontics, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China

[d]Guangdong Provincial Key Laboratory of Stomatology, Sun Yat-sen University, Guangzhou, Guangdong, P.R. China

[e]School of Materials Science and Engineering, South China University of Technology, Guangzhou, P.R. China

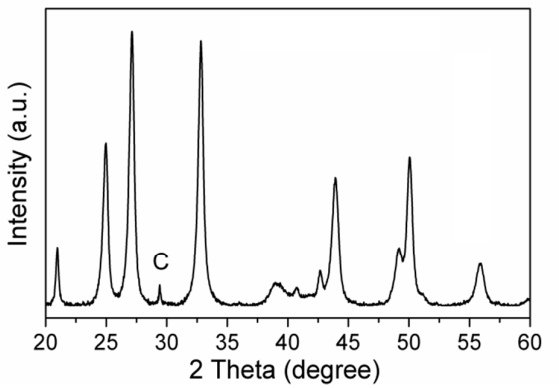

Figure S1. XRD pattern of the Casein/$CaCO_3$ microspheres fabricated by slowly adding Ca^{2+} solution into CO_3^{2-} solution (the concentration of casein was 2 mg/ml) using a syringe pump at 2 ml/min (mixing mode II). "C" represents XRD diffraction peak belonging to calcite phase, of which the relative intensity was significantly smaller than that of sample II-2.

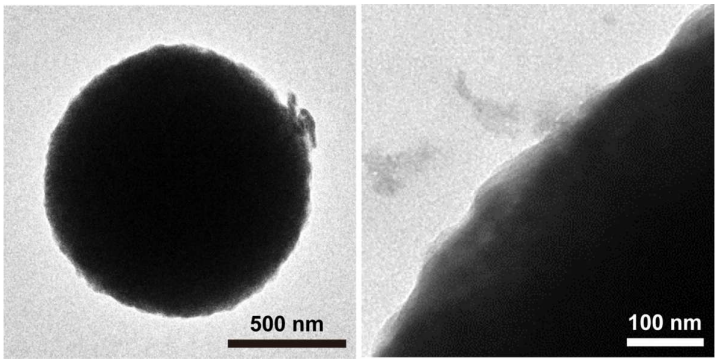

Figure S2. Representative TEM images of sample II-8. Compared with blank casein micelles, II-8 microspheres were much larger, more condensed and regular shaped with smoother surface.

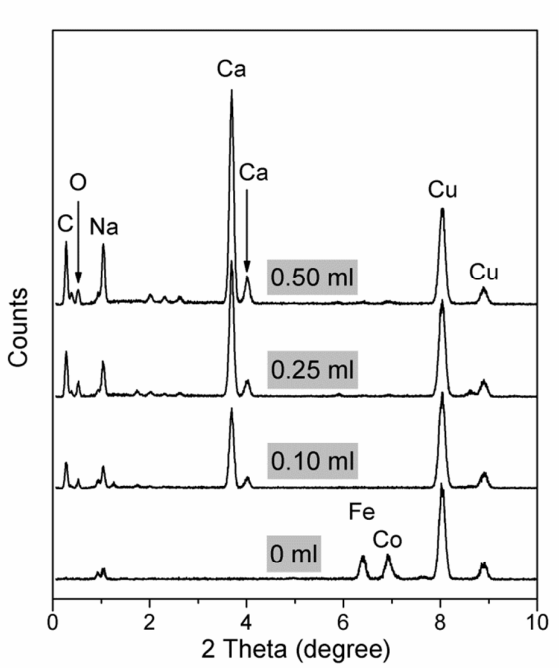

Figure S3. Representative EDX spectra of forming microspheres when different volumes of Ca^{2+} solution were added into 20 ml of CO_3^{2-} solution containing casein at 8 mg/ml under stirring (mixing mode II). After reaction for 20 min, samples were collected for TEM characterizations without centrifugation. Each spectrum was based on the signal collected from the center of forming microspheres. Fe, Co and Cu were either belonged to the copper grid which supported samples or the sample chamber in the TEM machine. For blank casein micelles (0 ml sample), limited by the resolution, only Na was detected.

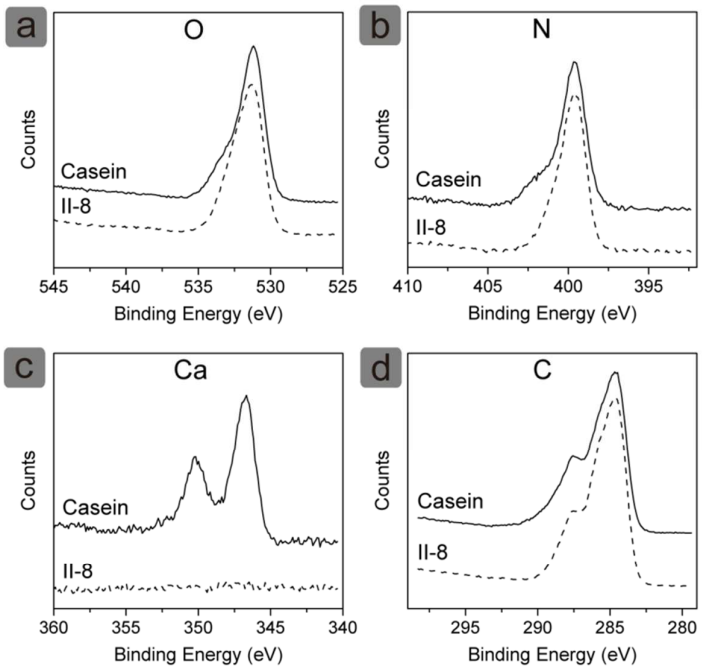

Figure S4. The XPS spectra of elements (a) O, (b) N, (c) Ca and (d) C in as-received casein and sample II-8. Ca was detected for II-8.

Table S1. Weight percentage of surface elements in as-received casein and sample II-8 based on XPS (Thermo Scientific Escalab 250Xi) analysis

Sample name	C (wt%)	Ca (wt%)	N (wt%)	O (wt%)	Weight percent of casein (wt%)
II-8	56.4	6.5	13.0	24.2	83.9
As-received casein	58.4	0	12.8	24.2	100

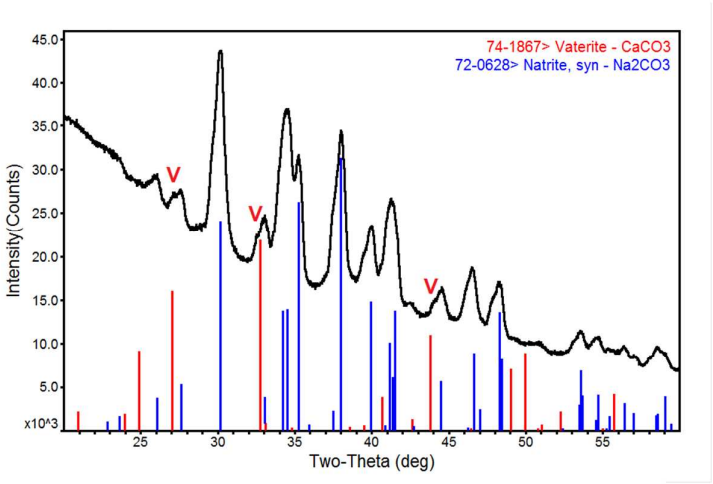

Figure S5. XRD pattern of the sample prepared by adding 1 ml of Ca^{2+} solution into CO_3^{2-} solution (20 ml, casein concentration at 8 mg/ml), collecting the suspension immediately without centrifugation and then lyophilization. Besides the diffraction peaks belonging to Na_2CO_3 (JCPDS# 72-0628), several small peaks of vaterite (JCPDS# 74-1867) were observed, as indicated by "V".

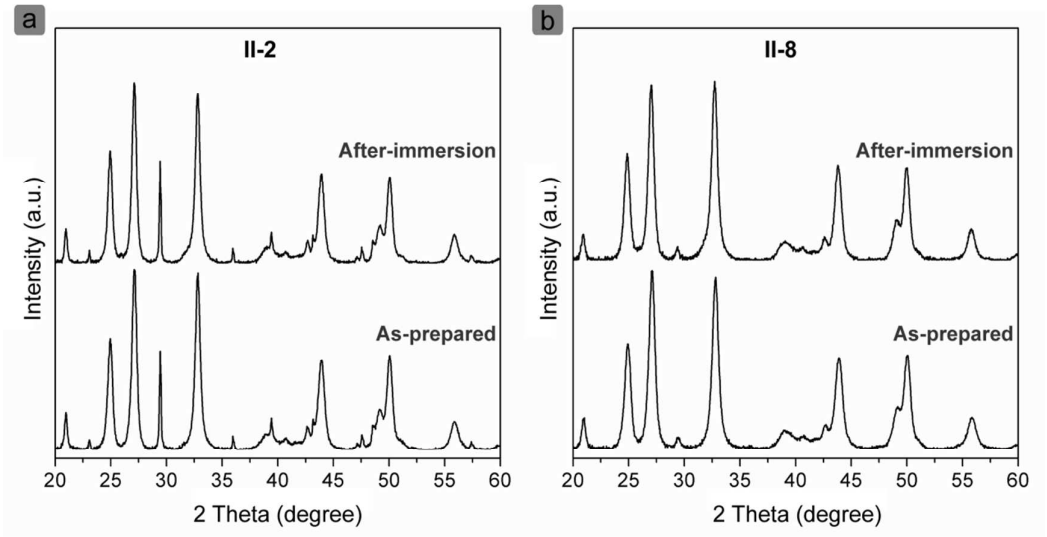

Figure S6. XRD patterns of as-prepared Casein/$CaCO_3$ microspheres and these microspheres after immersed in PBS (37°C) at 20 mg/ml for 24 hr. Sample (a) II-2 and (b) II-8 were selected as representatives. For each sample, the pattern of as-prepared microspheres was nearly the same as that of microspheres after immersion.

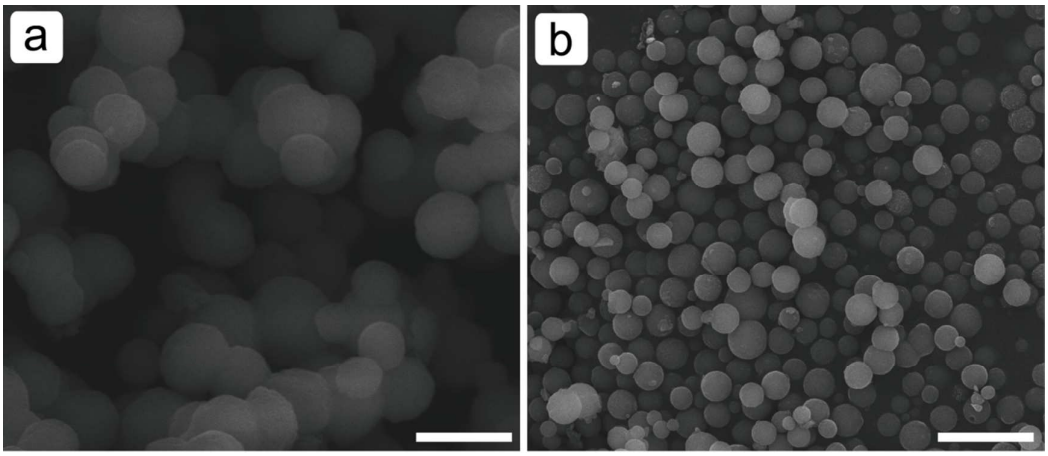

Figure S7. The representative SEM images of sample (a) II-2 and (b) II-8 after immersed in PBS (37°C) at 20 mg/ml for 24 hr. For each sample, morphology and microsphere size was nearly not affected by the immersion in PBS. Scale bar is 5 μm.

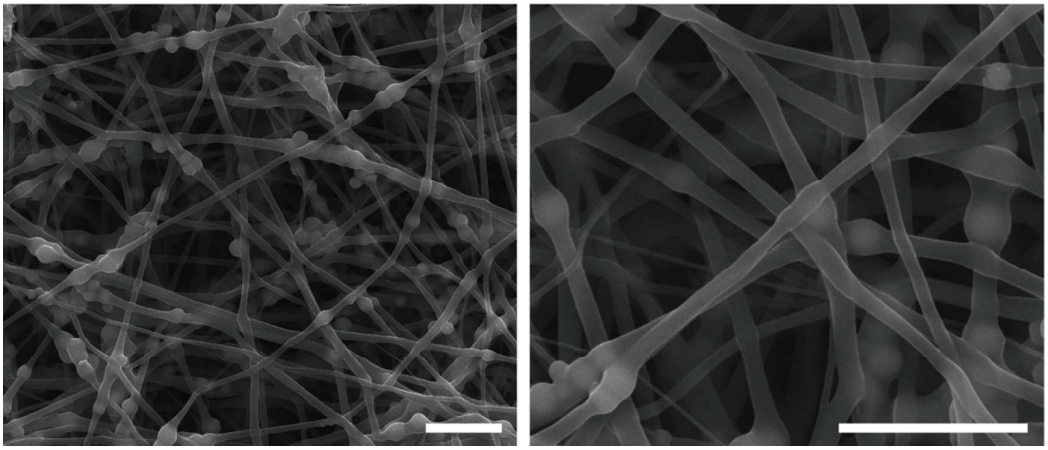

Figure S8. The representative SEM images of as-electrospun composite membrane PV. Casein/$CaCO_3$ microspheres were embedded in nanofiber matrix. Scale bar is 10 μm.