Electronic Supplementary Information for

Hierarchically Structured Nanocrystalline Hydroxyapatite Assembled Hollow Fibers as a Promising Protein Delivery System

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Experimental Section

***Electrospinning of BGF*:** The ultrafine BGF were synthesized using the electrospinning method reported previously. In a typical procedure, tetraethyl orthosilicate (TEOS, 1 mL), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, 0.378g), and triethyl phosphate (TEP, 0.1 mL) were dissolved in the mixture of 1M HCl solution (0.4 mL) and ethanol (6 mL). After stirring for 12h, poly(vinyl butyral) (PVB, M_w = 253 kDa, 0.5g) was added to this mixed solution under vigorous stirring for 6h to get the final transparent bioactive glass precursor. The final molar ratio of SiO_2:CaO:P_2O_5 was 70:25:5. Then the precursor solution was transferred into a syringe whose needle was connected to a high-voltage statitron. The feeding rate was kept at 0.6 mL h^{-1} using a syringe pump and 10 kV positive voltage was applied to a 10 cm gap between the nozzle and the rotating mandrel collector. Finally, the polymer was removed by calcining at 600℃ for 4h in air and the resulting BGF nonwoven membrane was obtained.

***Preparation of NHAHF*:** After cutting into suitable size, BGF membranes (~0.1g) were immersed in 0.2M $(NH_4)_2HPO_4$ aqueous solution in a Teflon-lined autoclave (50 mL). Then, the autoclave was sealed and heated at 150℃ for 0.5~4h in an oven. The obtained products could be directly picked up by tweezers without centrifugation or filtration process. The membrane was washed with deionized water and ethanol for several times and then dried in oven at 60℃ for 1 hour.

***Characterization*:** The structures and morphologies were tested by SEM (S-4800, Hitachi) and TEM (JEM-2100F, JEOL). XRD patterns were recorded using an X-ray diffractometer (D8 ADVANCE, Bruker AXS) with Cu (Kα) radiation at an operating condition of 40kV and 100mA. FTIR spectra were obtained on a FTIR spectrometer (Nicolet 380, Thermo Scientific). The element contents were characterized by EDS (JXA-8100, Oxford Instruments). Nitrogen sorption isotherms were measured on a Micrometitics Tristar 3000 system.

***Protein Loading and Release*:** For protein loading, the HA/BGF, HA/PBGF, and NHAHF (5 mg) were immersed in PBS (1.5 mL, pH 7.4) containing variable BSA concentrations (0-4000 μg mL^{-1}) for 6h at 37℃. The amount of BSA in the PBS before and after adsorption process was measured by BCA protein assay kit (Pierce, Thermo Scientific). The amount of BSA loading was calculated using the formula: BSA loading (mg g^{-1}) = $1.5(C_a-C_b)/W_s$, where C_a and C_b are the BSA concentration in PBS before and after immersion, respectively, and W_s the sample weight.

After loading the maximum amount of BSA, these three samples were then immersed in PBS (1.5 mL, pH 7.4) and incubated at 37℃ in a thermostat shaker to investigate the release kinetics of BSA. At predetermined time intervals, 50μL incubated PBS was removed for monitoring by BCA protein assay kit and replaced with fresh PBS. The cumulative release dose was calculated based on the standard curve and the data are expressed as mean ± standard deviation.

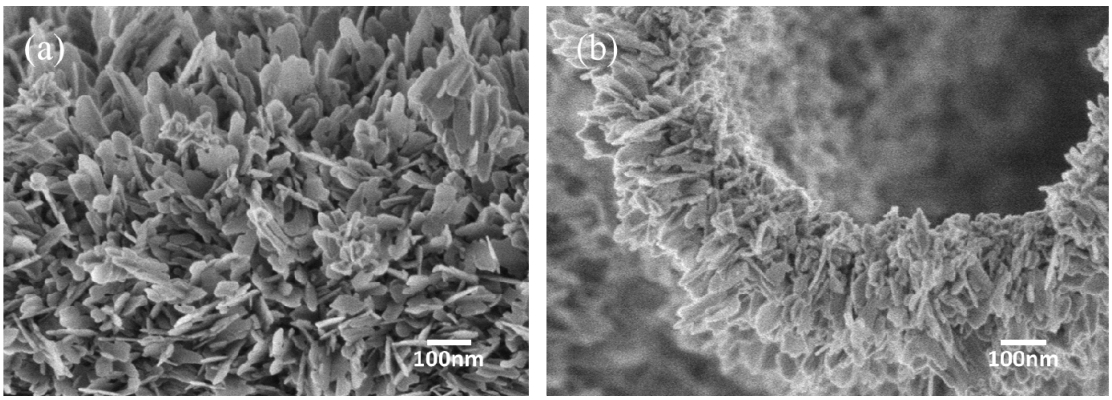

Figure S1. Ultrahigh-magnification SEM images of the external surface (a) and the cross section (b) of NHAHF

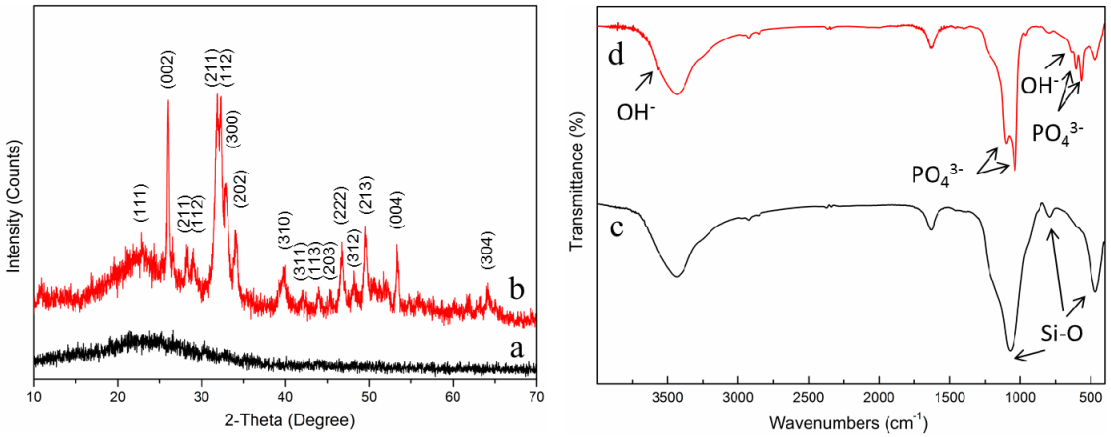

Figure S2. XRD patterns and FTIR spectra of BGF (a,c) and NHAHF (b,d): Before hydrothermal reaction, the BGF were in the amorphous state and showed the characteristic absorption bands of Si-O bond at 470, 800, 1080 cm^{-1} which were consistent with observations described previously.[1a] After hydrothermal treatment for 2 hours, the diffraction peaks appeared in the XRD pattern could be well assigned to corresponding crystal planes of HA (JCPDS No. 09-0432). Concurrently, the absorption bands related to the silica glass in FTIR spectrum were substantially attenuated, while bands attributed to the phosphate groups (570, 605, 960, 1032, 1100 cm^{-1}) and the apatitic hydroxyl groups (3572, 631 cm^{-1}) appeared,[2] suggesting that the plate-like building blocks of the hollow fibers were HA nanocrystals. Besides, it can be observed that the relative intensity of the (002) face of HA in the XRD pattern is extraordinarily strong which indicates that the *c*-axes of HA nanocrystals have a preferred orientation normal to the fiber external surface.[3]

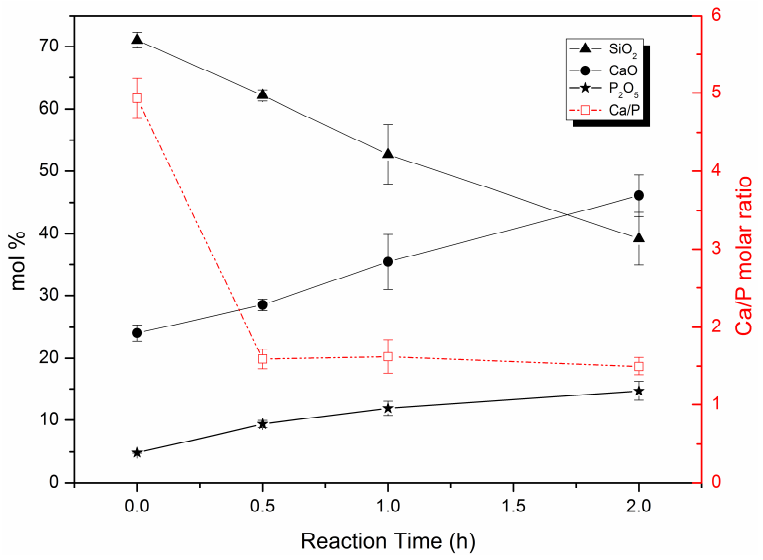

Figure S3. The variation of the composition and Ca/P molar ratio of the fibers with hydrothermal reaction time

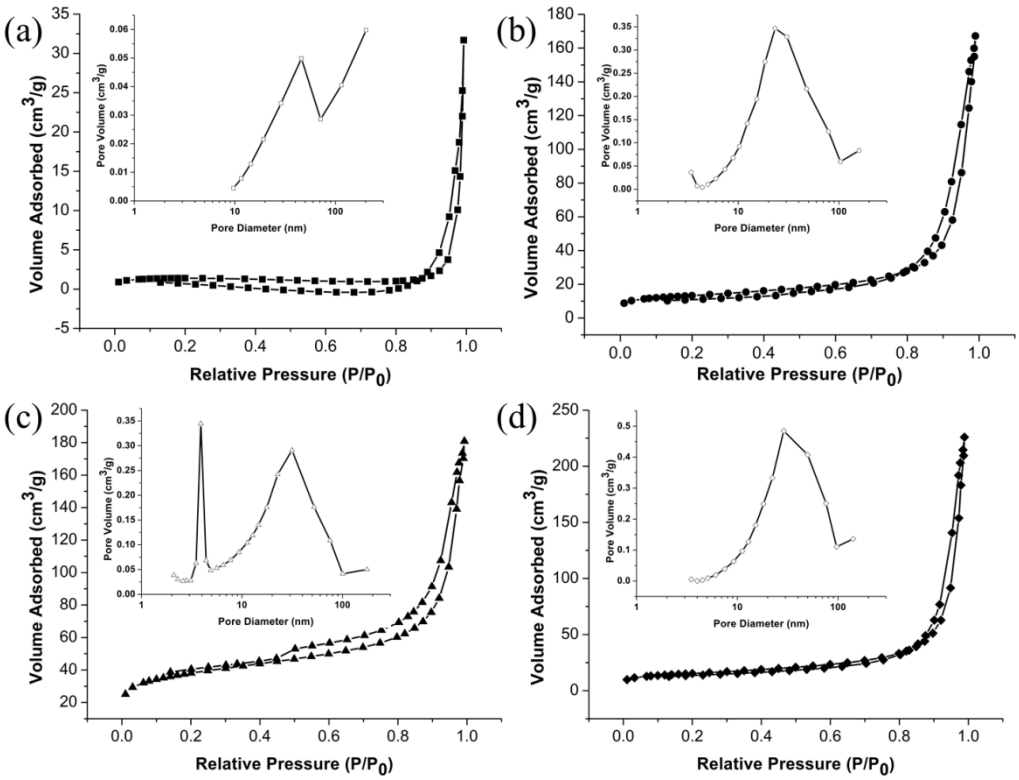

Figure S4. Nitrogen adsorption-desorption isotherms and BJH-desorption pore size distributions of BGF (a), HA/BGF (b), HA/PBGF (c), NHAHF (d)

Table S1. The BET specific surface area (S_{BET}), BJH-desorption cumulative pore volume (Vp), and BSA Loading Capacity of the membranes

Sample	S_{BET} [$m^2\ g^{-1}$]	V_p [$cm^3\ g^{-1}$]	BSA Loading Capacity	
			m_s [$mg\ g^{-1}$]	n_s [$mg\ m^{-2}$][a]
BGF	4.8	4.2	-	-
HA/BGF	46.4	44.98	45.8	0.99
HA/PBGF	133.6	77.72	68.8	0.51
NHAHF	53.9	49.73	96.8	1.80

[a] $n_s = m_s/S_{BET}$

References:

1 (a) W. Xia, D. Zhang and J. Chang, *Nanotechnology*, 2007, **18**, 135601; (b) H. W. Kim, H. E. Kim and J. C. Knowles, *Adv. Funct. Mater.*, 2006, **16**, 1529.
2 S. Koutsopoulos, *J. Biomed. Mater. Res.*, 2002, **62**, 600.
3 D. Liu, K. Savino and M. Z. Yates, *Adv. Funct. Mater.*, 2009, **19**, 3941.