Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

for Journal of Materials Chemistry B

Enhancing *in vitro* bioactivity and *in vivo* osteogenesis of organic-inorganic nanofibrous biocomposites with novel bioceramics

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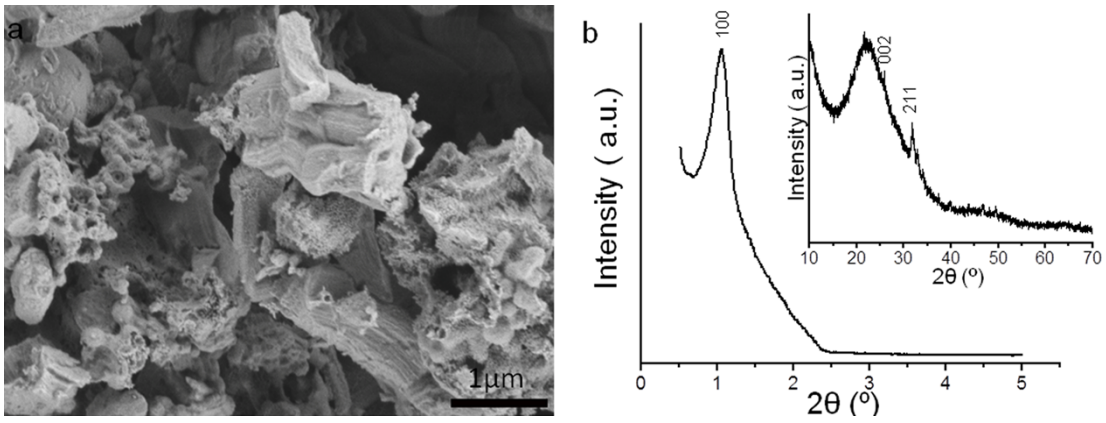

Figure S1. (a) FESEM image of MGHA nanocomposite; (b) Small-angle XRD pattern of MGHA nanocomposite showing the ordered mesoporous arrangement, (inset) Wide-angle XRD pattern demonstrating the presence of the apatite phase

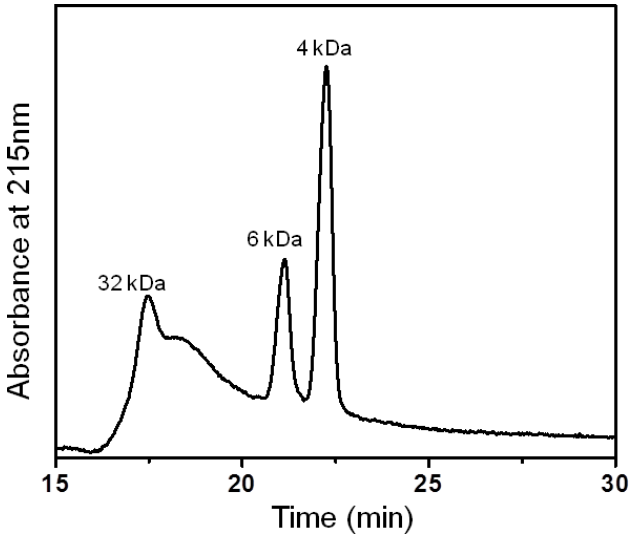

Figure S2. The characteristic MW distribution of regenerated SF by dissolution in $CaCl_2/H_2O/EtOH$

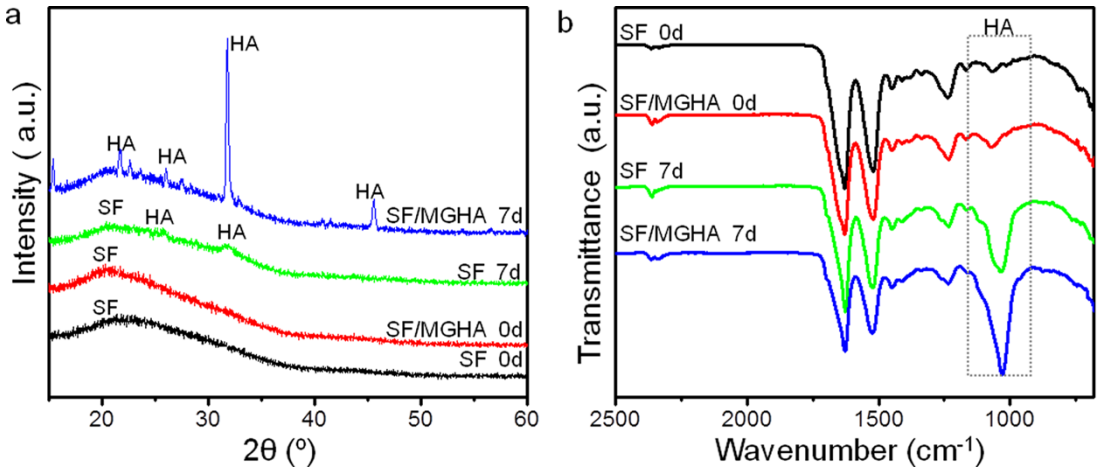

Figure S3. (a) Wide-angle patterns of pure SF and SF/MGHA before and after soaking in SBF; (b) ATR-FTIR spectra of pure SF and SF/MGHA before and after soaking in SBF

Considering the effect of preparing the SF solution on the cell reponse, osteoblast-like cell MG-63 was cultured on the SF and SF/MGHA mats to evulate the *in vitro* biocompatibility. After seeding on material, the cell/material interactions including adhesion, proliferation and differentiation were proposed. Figure S4 shows the representative FESEM images of MG-63 cultured on the electrospun mats at 2 d. It was found that the cells attached well on the pure SF and SF/MGHA composite mats, and the spreading cells maintained physical contact with each other through filopodia.

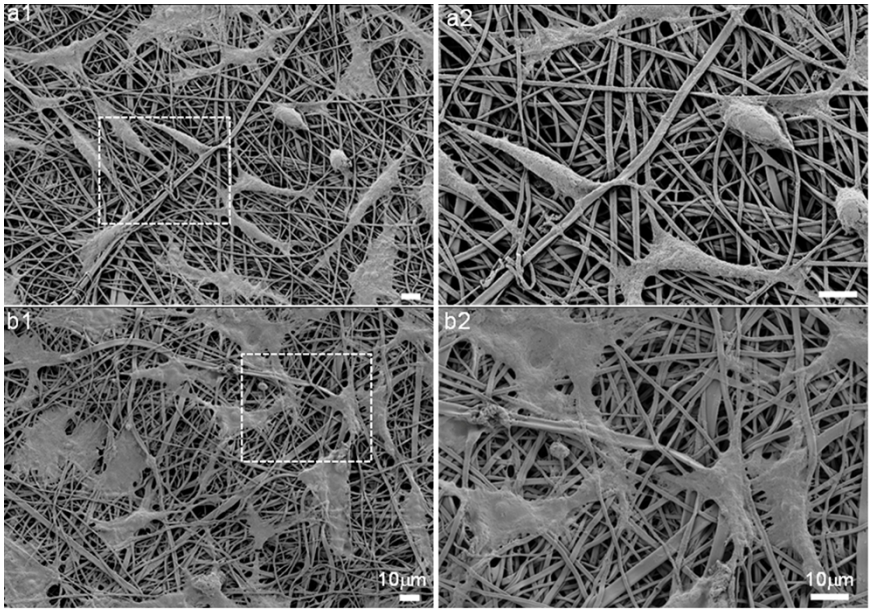

Figure S4. Morphology of MG-63 cells on pure SF and SF/MGHA nanofibrous membranes for 2 d (a1–a2: pure SF; b1–b2: SF/MGHA)

In the Figure S5, cell proliferation (MTT assay) and cell differentiation (ALP activity) for viability of MG-63 were measured after the cells cultured on the mats at each time point. For MTT assay (Figure S5a), MG-63 cells continued to proliferate with the increase of culture time. Particularlly in the SF/MGHA nanofibers, the cells proliferated more actively as compared to pure SF, indicating that the presence of MGHA was conducive to promote the proliferation of osteoblasts. For ALP activity (Figure S5b), the difference of ALP activity between pure SF and SF/MGHA was not significant at 3 d ($p > 0.05$), but showed a significant stimulation of ALP production at both 6 d and 9 d on the SF/MGHA composite when compared with pure SF mats. These above

results indicated that according to the method of SF solution or spinning dope, the obtained molecular weight of SF may not affect the cell response. The presence of MGHA powders in SF matrix may accelerate the cell attachment on the membrane, furthermore promoted the cell proliferation and differentiation making it the good biocompatibility.

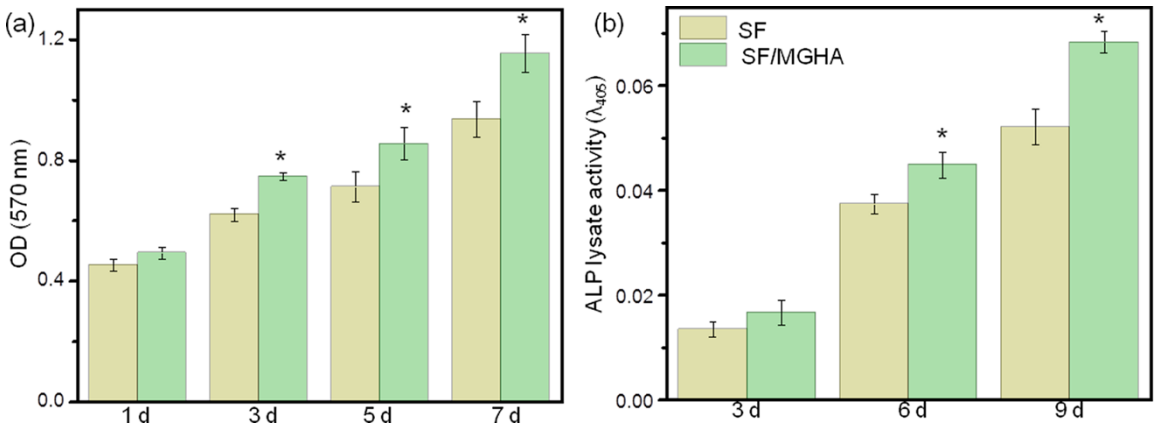

Figure S5. (a) MTT assay for viability of MG-63 cultured on the pure SF and SF/MGHA composite scaffolds for 1d, 3 d, 5 d, and 7 d, respectively; (b) ALP activity expressed by MG-63 cultured on the pure SF and SF/MGHA composite mats at each time point (* indicates significant difference compared to pure SF group, $p < 0.05$).

Supporting Method for *in vitro* osteoblast-like cell (MG-63) responses on the electrospun SF and SF/MGHA mats

Cell culture assay: These electrospun SF and SF/MGHA mats used with cell cultures were sterilized with 75% EtOH and ultraviolet (UV) light, and then were immerged in a culture medium overnight. Osteoblast-like cells MG-63 were used to observe cellular behavior in the scaffolds. MG-63 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were maintained up to passage 5 and collected via trypsin EDTA treatment. The cells were then seeded onto the mats at a density of 1×10^5 cells per sample and incubated in an atmosphere of 5% CO_2 at 37 °C. The medium

was changed every second day.

Cell morphology: To assess the morphology of cells on the scaffolds, the cells were examined by FESEM after 2 days. The cell/scaffold constructs were fixed in 2.5% glutaraldehyde and dehydrated through a graded ethanol series. Dried scaffolds were coated with gold and examined under SEM.

MTT assay: MTT assay was used to quantitatively assess the number of viable cells attached and grown on the mats. Briefly, after being cultured for 1d, 3 d, 5 d, and 7 d, respectively, the culture medium was removed, and then 1 mL serum-free medium and 100 mL MTT (Sigma) solution (5 mg mL^{-1} in PBS) were added into each well, followed by incubation at 37 °C for 4 h for MTT formazan formation. The upper solvent was then discarded and the blue formazan reaction product was dissolved by adding 200 mL DMSO. The dissolvable solution was transferred to a 96-well plate. During the dissolving process, the mats were squeezed to ensure the complete extraction of the formazan. The optical density at 570 nm (OD_{570}) was determined against the sodium dodecyl sulfate solution blank using a spectrophoto-metric microplate reader ((Model 680, Bio-Rad). The data were reported as the mean of five examinations.

ALP activity: For MG63 cells seeded in the mats for 3 d, 6 d, and 9 d, alkaline phosphatase (ALP), which is a marker of osteoblast activity, was assayed by measuring the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (*p*-NPP). The mats seeded with MG-63 cells were rinsed gently with PBS (phosphate-buffered saline), and incubated in Tris buffer (10 mM, pH 7.5) containing 0.1% Triton X-100 for 10 min. Next, 100 μL of the lysate was added to a 96-well plate containing 100 μL of *p*-NPP solution, which was prepared using an ALP kit (Beyotime Institute of Biotechnology). The ALP activity was determined by measuring the absorbance at 405 nm, using a microplate reader (SpectraMax M5).