## Supporting Information

## Injectable Hydrogel Systems with Multiple Biophysical and

**Biochemical Cues for Bone Regeneration** 

Weinan Cheng<sup>a,b,c,#</sup>, Zhaozhao Ding<sup>b,#</sup>, Xin Zheng<sup>d,#</sup>, Qiang Lu<sup>a,b,\*</sup>, Xiangdong Kong<sup>e</sup>,

Xiaozhong Zhou<sup>a,\*</sup>, Guozhong Lu<sup>b,\*</sup>, David L Kaplan<sup>f</sup>

<sup>a</sup>Department of Orthopedics, The Second Affiliated Hospital of Soochow University,

Suzhou 215000, People's Republic of China

<sup>b</sup>Department of Burns and Plastic Surgery, The Affiliated Hospital of Jiangnan University, Wuxi 214041, People's Republic of China

<sup>c</sup>Department of Orthopedics, The First Affiliated Hospital of Xiamen University, Xiamen 361000, People's Republic of China

<sup>d</sup>Department of Orthopedics, Taizhou Municipal Hospital, Taizhou 318000, People's Republic of China

<sup>e</sup>Zhejiang-Mauritius Joint Research Center for Biomaterials and Tissue Engineering, School of Materials Science and Engineering, Zhejiang Sci-Tech University,

Hangzhou 310018, People's Republic of China

<sup>f</sup>Department of Biomedical Engineering, Tufts University, Medford, Massachusetts 02155, United States

## **Corresponding author:**

Qiang Lu, Tel: (+86)-512-67061649; E-mail: Lvqiang78@suda.edu.cn

Xiaozhong Zhou, E-mail: Zhouxz@suda.edu.cn

Guozhong Lu, E-mail: Luguozhong@hotmail.com

**Keywords:** Injectable hydrogel; Silk nanofiber; Bone regeneration; Osteogenic cues; Niches



**Fig. S1** Representative images of cells and flow cytometry analysis of CD marker expression in third-passaged BMSCs: (a, b) Morphology of third-passaged BMSCs (P3). Scale bars: 200  $\mu$ m (a) and 100  $\mu$ m (b), respectively. (c) The expression of CD90 (95.2%), CD44 (94.5%), CD45 (1.7%) and CD34 (12%) in the BMSCs. FITC-conjugated mouse IgG1 was used as a negative control.



**Fig. S2** The micromorphologies of different silk nanofibers (SNF) and hydroxyapatite nanoparticles (HA): AFM images of (a) SNFs, (b) DFO-loaded SNFs, (c) BMP-2-loaded SNFs, (d) HA and (e) BMP-2-loaded HA. Scale bar, 500 nm. (f, g): SEM images of HA and BMP-2-loaded HA nanoparticles respectively. Scale bar, 500 nm.



Fig. S3 Osteogenesis of BMSCs on different composite hydrogels measured by immunofluorescence assay: (a) OCN expression at day 14. Scale bar, 50  $\mu$ m. (b, c) mRNA levels of RUNX2 and OCN detected by real time PCR at day 14, respectively. \*P $\leq$ 0.05, \*\*P $\leq$ 0.01, and \*\*\*P $\leq$ 0.001.



**Fig. S4** Masson's trichrome staining of the defects treated with different composite hydrogels at 1 week, 2 weeks, 4 weeks and 8weeks after the implantation. Scale bar, 20X: 1 mm, 200X: 100 μm.