

Supporting information

# **Mesenchymal stem cell-loaded thermosensitive hydroxypropyl chitin hydrogel combined with a three-dimensional-printed poly( $\epsilon$ -caprolactone) /nano-hydroxyapatite scaffold to repair bone defects via osteogenesis, angiogenesis and immunomodulation**

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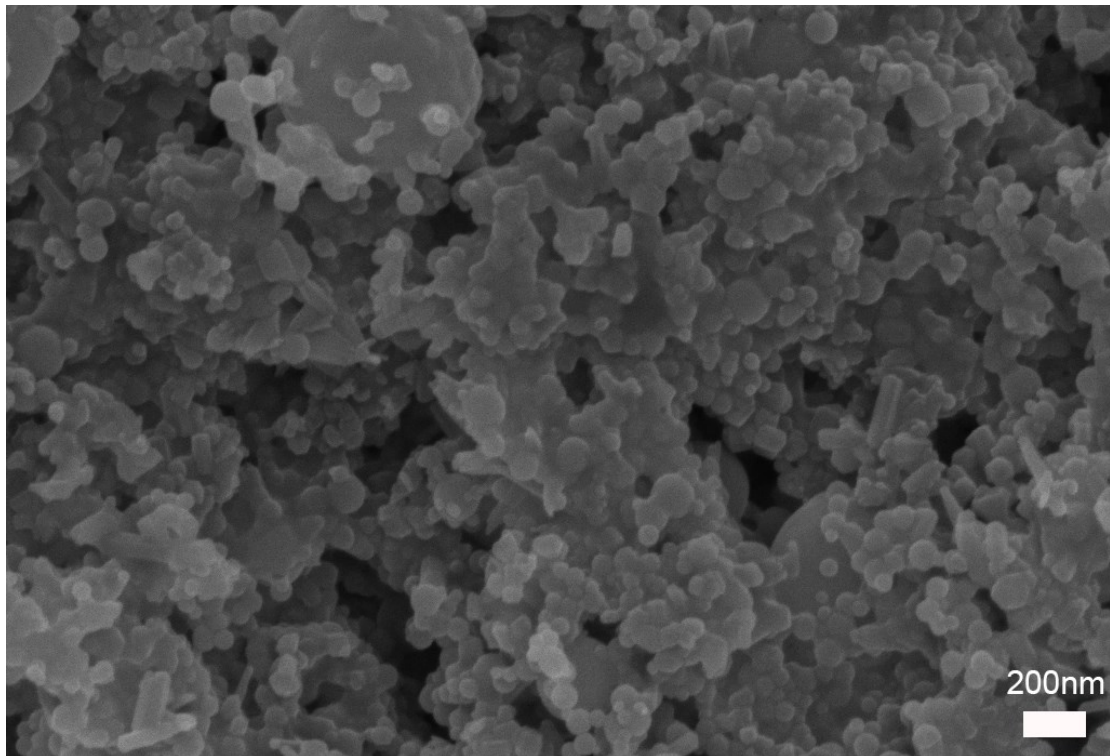
**Table S1.** Primers used in Real-Time PCR for RAW 264.7.

<b>Gene</b>	<b>Forward primer sequence(5'-3')</b>	<b>Reverse primer sequence(5'-3')</b>
<b>GADPH</b>	TTCCAGGAGCGAGACCCCACTA	GGGCGGAGATGATGACCCTTTT
<b>BMP2</b>	GGGACCCGCTGTCTTCTAGT	TCAACTCAAATTCGCTGAGGAC
<b>TGF <math>\beta</math>1</b>	CCACCTGCAAGACCATCGAC	CTGGCGAGCCTTAGTTTGGAC
<b>Ptges</b>	GGATGCGCTGAAACGTGGA	CAGGAATGAGTACACGAAGCC
<b>VEGF<math>\alpha</math></b>	TATTCAGCGGACTCACCAGC	AACCAACCTCCTCAAACCGT
<b>PDGFB</b>	CATCCGCTCCTTTGATGATCTT	GTGCTCGGGTCATGTTCAAGT
<b>MMP9</b>	GCAGAGGCATACTTGTACCG	TGATGTTATGATGGTCCCCTTG
<b>IL1<math>\beta</math></b>	CCCAACTGGTACATCAGCACCTC	GACACGGATTCCATGGTGAAGTC
<b>TNF-<math>\alpha</math></b>	GGACTAGCCAGGAGGGAGAA	CGCGGATCATGCTTTCTGTG
<b>IL6</b>	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
<b>Arg-1</b>	CTCCAAGCCAAAGTCCTTAGAG	GGAGCTGTCATTAGGGACATCA
<b>IL10</b>	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
<b>CCL22</b>	CTCTGCCATCACGTTTAGTGAA	GACGGTTATCAAAAACAACGCC

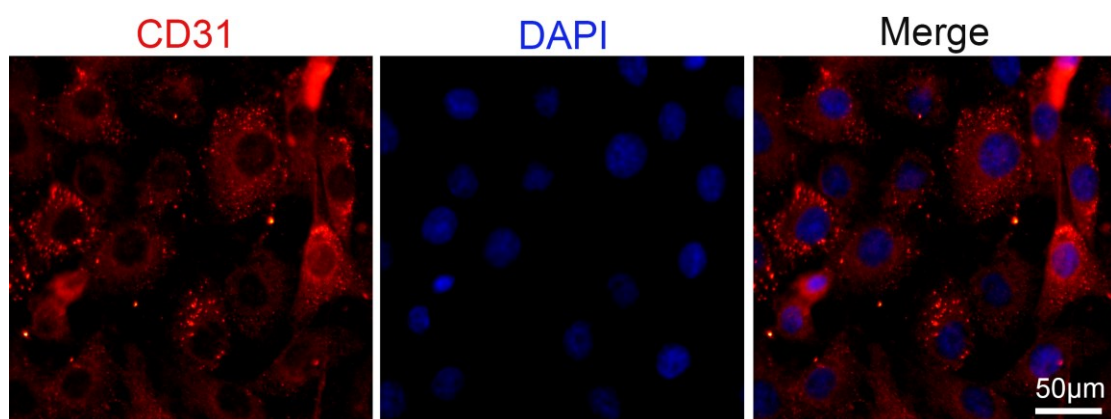
**Table S2.** Primers used in Real-Time PCR for MSCs.

<b>Gene</b>	<b>Forward primer sequence(5'-3')</b>	<b>Reverse primer sequence(5'-3')</b>
<b>GADPH</b>	GGTGGACCTCATGGCCTACA	CTCTCTTGCTCTCAGTATCCTTGCT
<b>OCN</b>	GCCCTGACTGCATTCTGCCTCT	TCACCACCTTACTGCCCTCCTG
<b>OPN</b>	CCAGCCAAGGACCAACTACA	GCTGGCAGTGAAGGACTCAT
<b>ALP</b>	GCACAACATCAAGGACATCG	TCAGTTCTGTTCTTGGGGTACAT
<b>Runx 1</b>	TGCGTATCCCCGTAGATGCC	GTGGTCAGCTAGTACCTCCAC
<b>Runx 2</b>	GGGACCGACACAGCCATATA	TCTTAGGGTCTCGGAGGGAA
<b>Runx 3</b>	TGTAACACCAAGCACACCCA	GTTCAGGTCTGAGGAGCCTTG
<b>COL I</b>	GGCAGATGGTGGATGGTAAGT	CCAGACTCTTCAAACCTCGCT
<b>COL X</b>	TGCTGCTAGTGTCTTGACG	CCTCTTACTGAAATCTCTTTACCCT

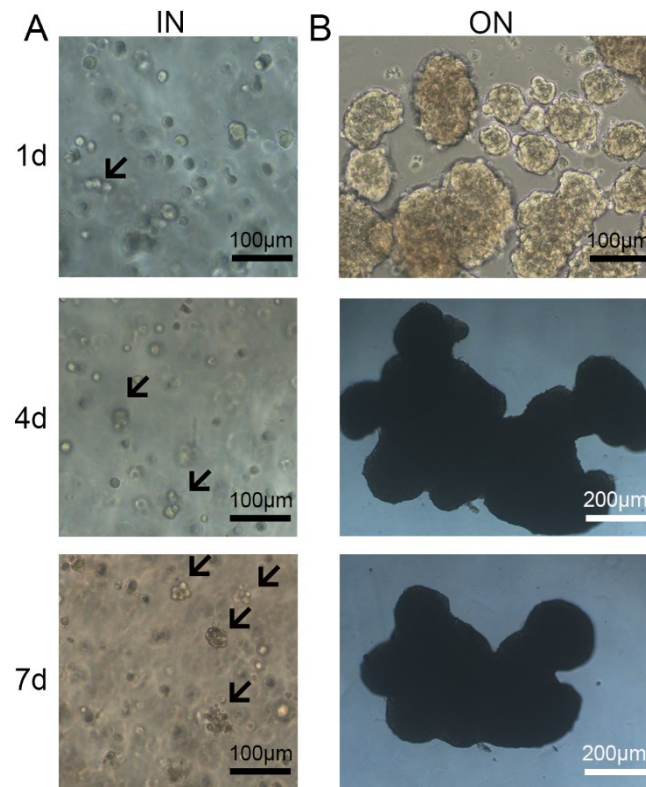
**Fig.S1** The SEM characterization of nano-hydroapatite(nHA). Scale bar, 200 $\mu$ m.



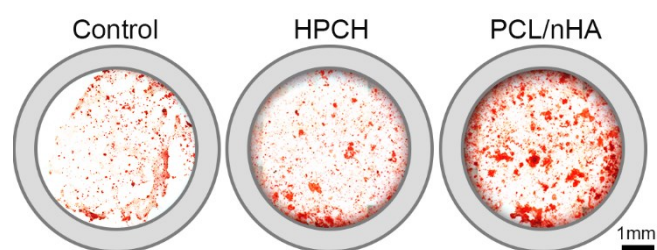
**Fig.S2** The identification of rat endothelial cells using immune-fluorescent staining of CD31. Scale bar, 50 $\mu$ m.



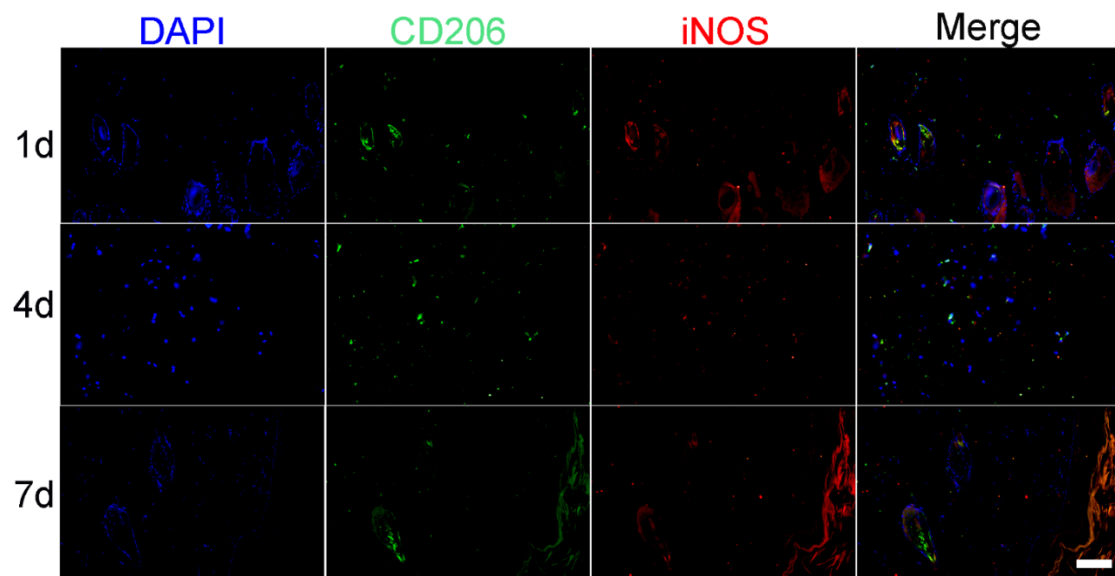
**Fig.S3** MSC spheroids formation in or on HPCH. MSCs encapsulated in (A) HPCH hydrogel and on (B) HPCH hydrogel after 1 day, 4 days and 7 days' culture.



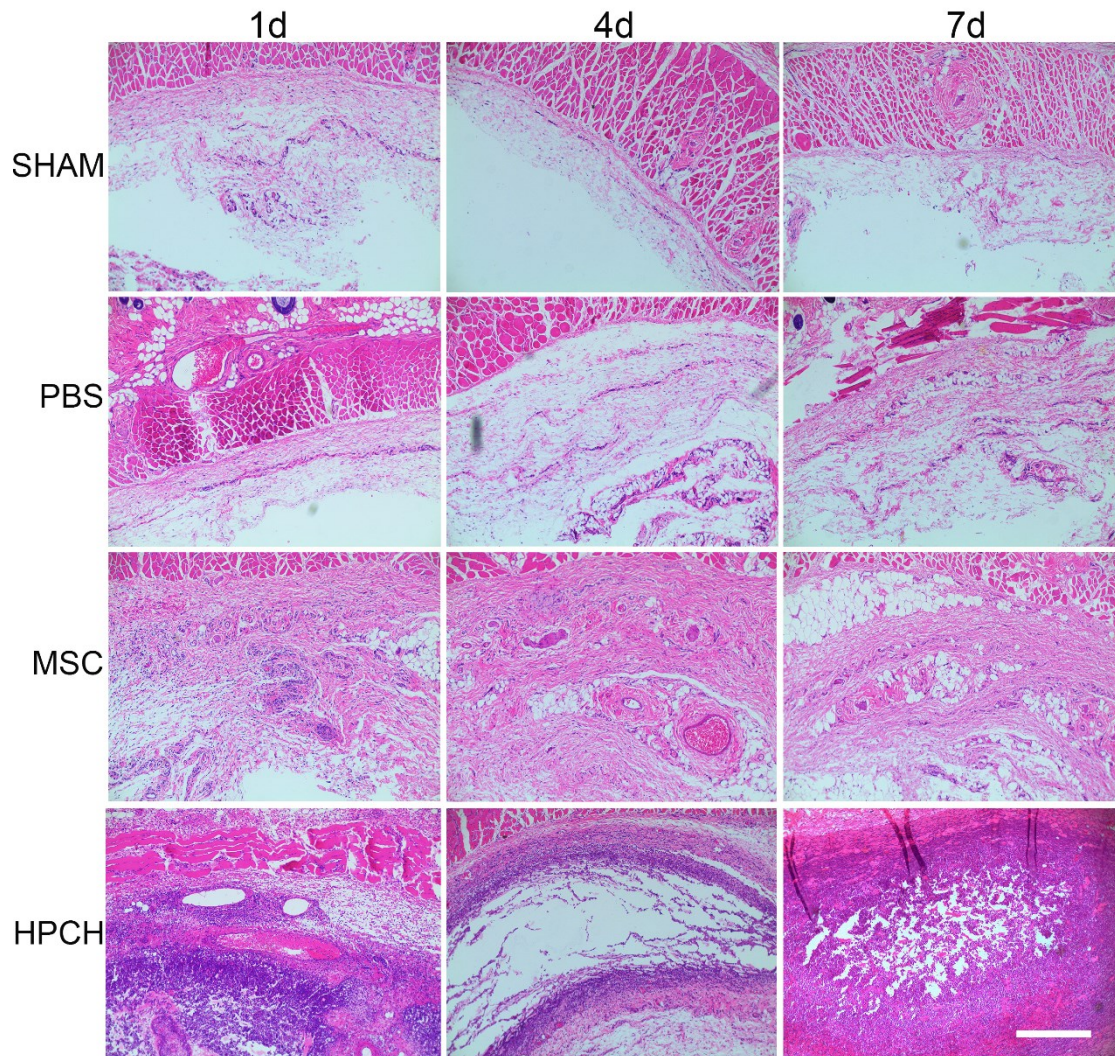
**Fig. S4** Alizarin red staining of MSCs for 14 days' culture. The culture medium was the extract medium from RAW 264.7 cultured with PCL/nHA or HPCH. Scale bar, 1mm.



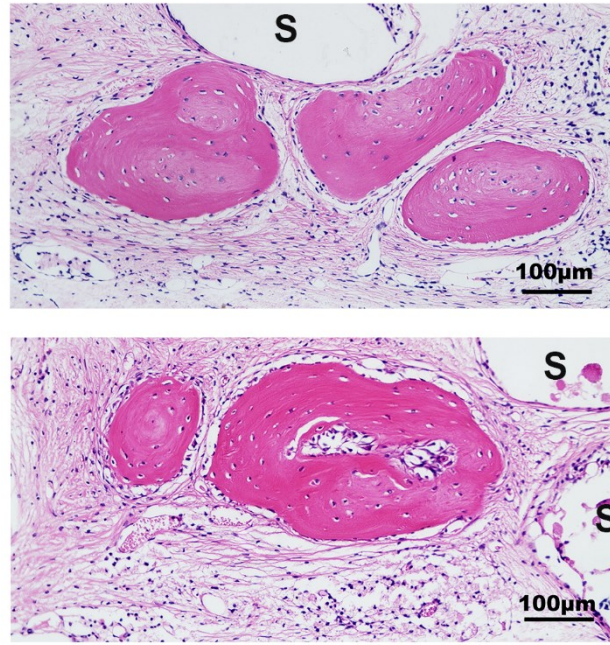
**Fig.S5** Immunofluorescent staining of the subcutaneous injection of MSCs. Scale bar, 100  $\mu\text{m}$ .



**Fig. S6** H&E staining for the subcutaneous implantation of MSCs and HPCH for 1d, 4d and 7d. Scale bar, 400  $\mu$ m.



**Fig.S7** New bone formed in the central of the scaffold. Concentrically arranged lamellae of bone matrix and osteocytes lived within lacuna, and marrow cavity was formed in the “osteon”. S, scaffold. Scale bar, 100μm.



**Video 1.** 3D reconstruction of PCL/nHA based on micro-CT result.