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

Supporting Information (SI)

A dual-delivery system of pH-responsive chitosan-functionalized mesoporous silica nanoparticles bearing BMP-2 and dexamethasone for enhanced bone regeneration

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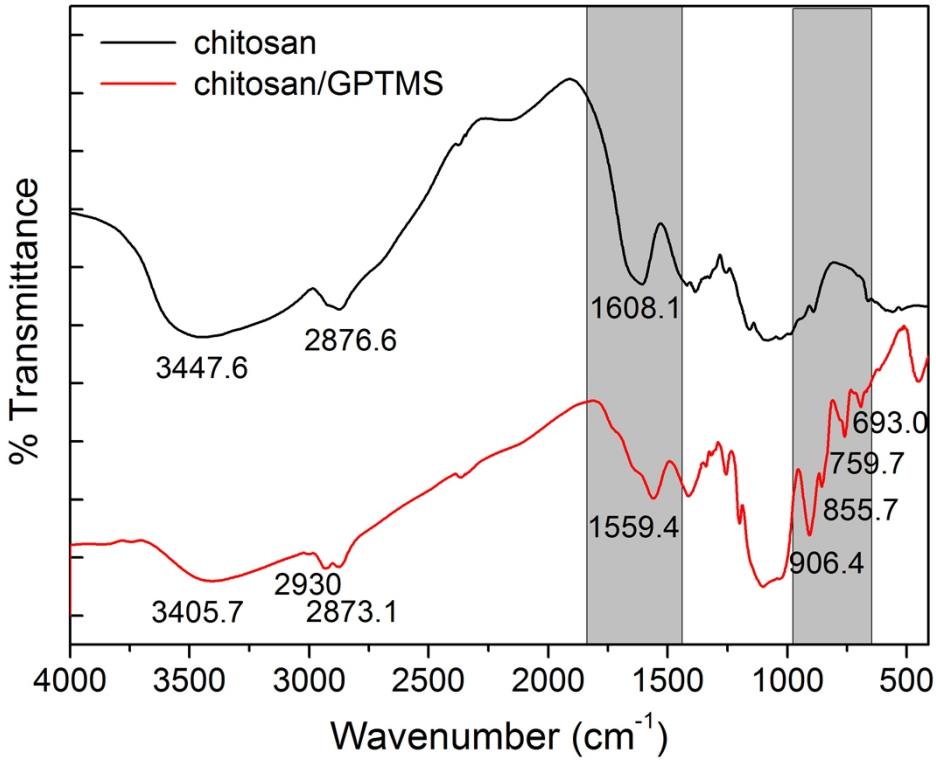

Fig. S1 FTIR spectra of chitosan and chitosan/GPTMS. The peak centered at 1608.1 cm^{-1} was assigned to the characteristic stretching vibrations of the primary amine (–NH_2 bond) in the main chain of chitosan. However, in the FTIR spectra of chitosan/GPTMS, a band at 1559.4 cm^{-1} was appeared. This peak was corresponding to the group of secondary amine (–NH– bond), which supported the successful introduction of epoxy and amine groups. The peaks presented between 600 – 900 cm^{-1} was attributed to the stretching vibrations of C-Si bond, which confirmed again that GPTMS was linked to the main chain of chitosan.

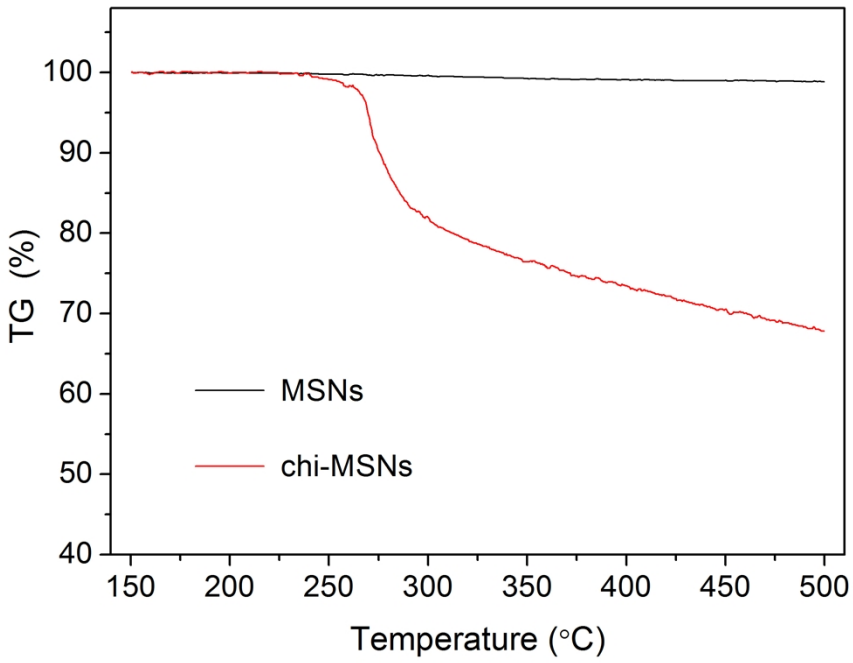

Fig. S2 The themogravimetric analysis of MSNs and chi-MSNs.

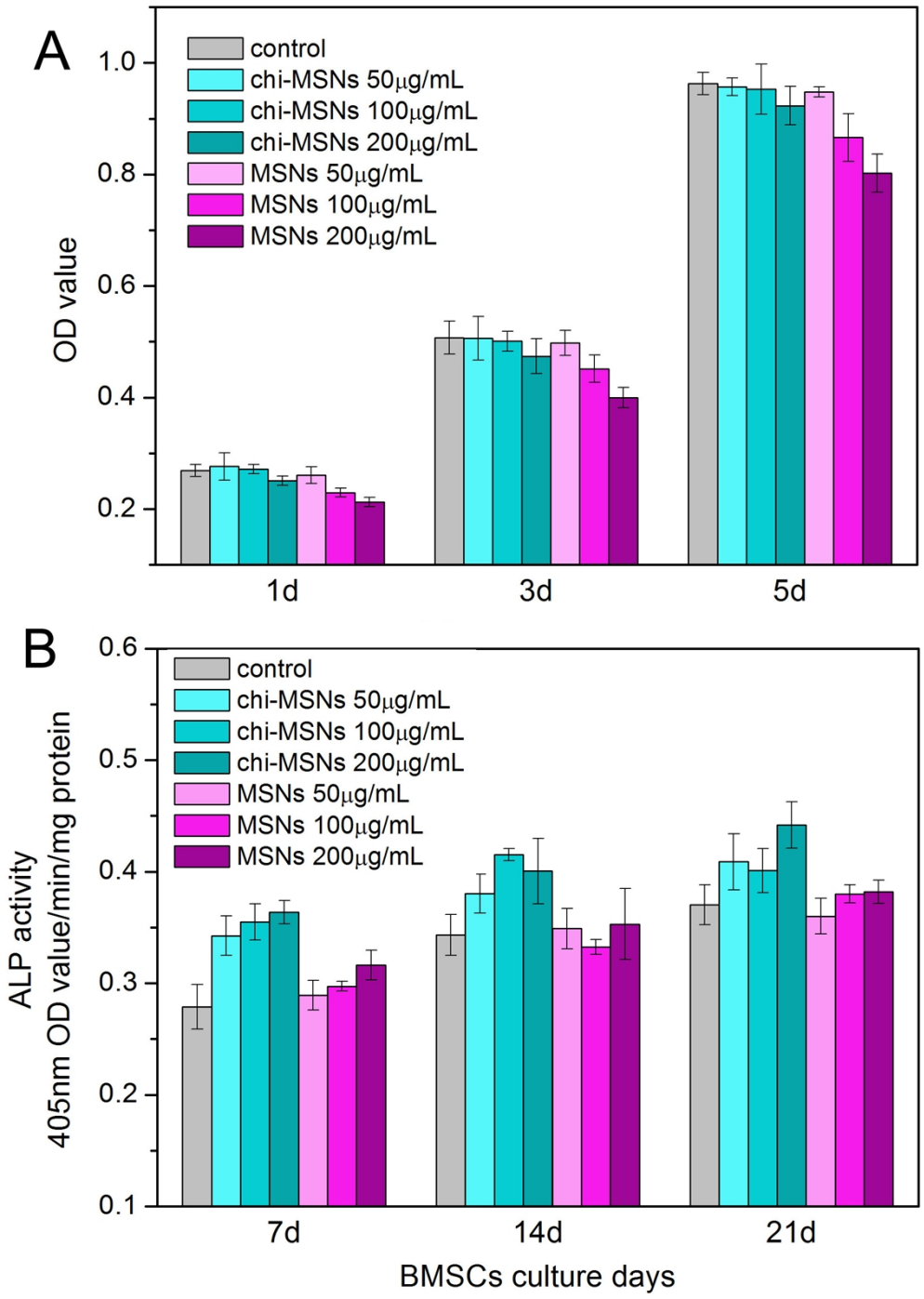

Fig. S3 (A) *In vitro* cell proliferation of MSNs and chi-MSNs towards bMSCs at day 1, 3 and 5, determined by MTT assay. (B)ALP activity of bMSCs incubated with MSNs and chi-MSNs for 7, 14 and 21 days. The concentrations of MSNs and chi-MSNs are set as 50, 100 and 200 μg/mL.

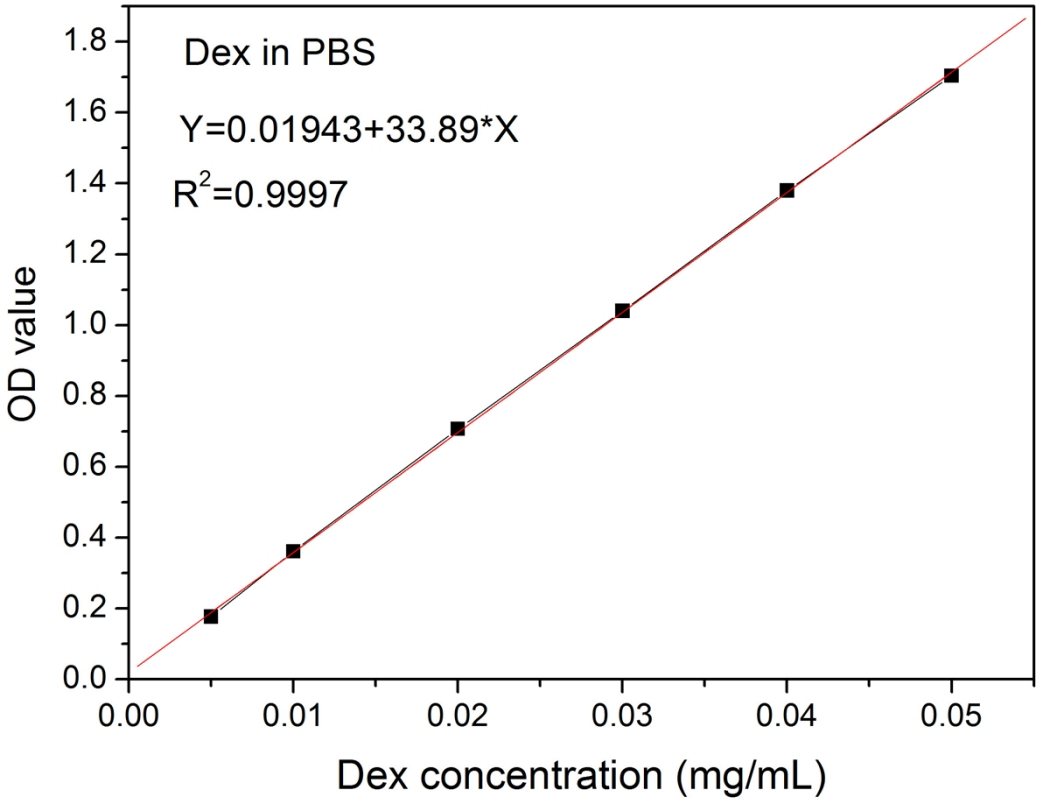

Fig. S4 Calibration curve of dexamethasone in PBS.

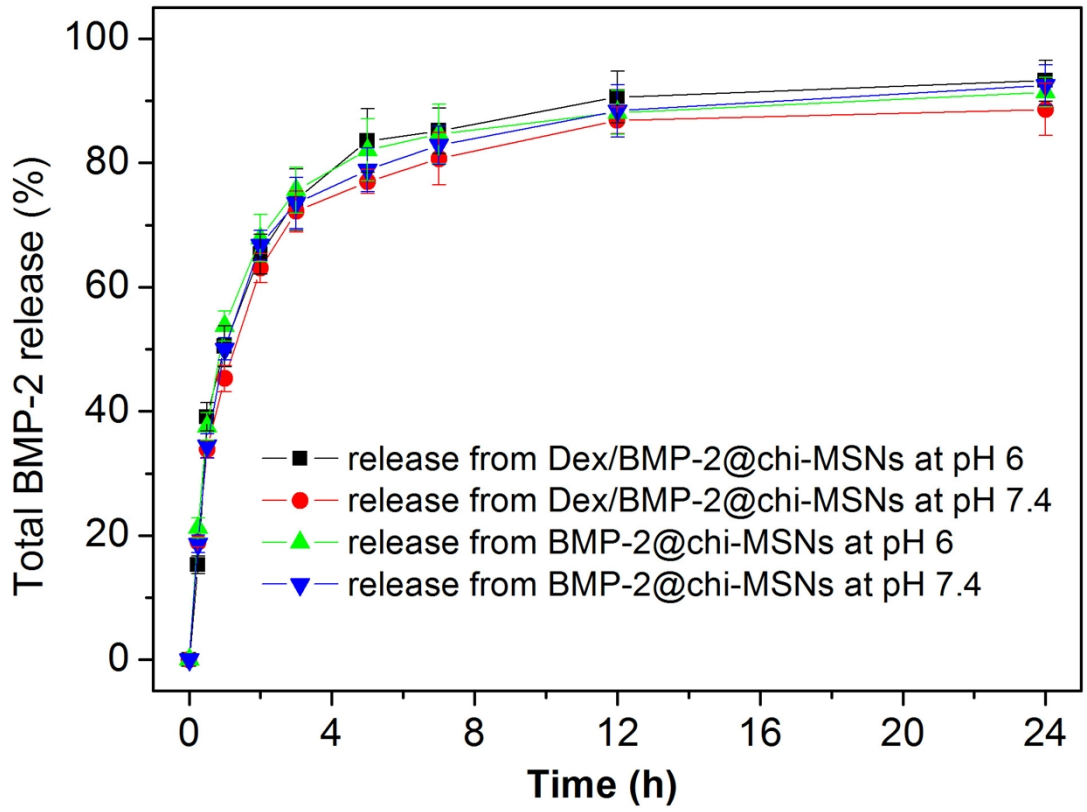

Fig. S5 Cumulative release of BMP-2 from Dex/BMP-2@chi-MSNs and BMP-2@chi-MSNs in phosphate buffer solution (PBS) at pH 6.0 and 7.4.

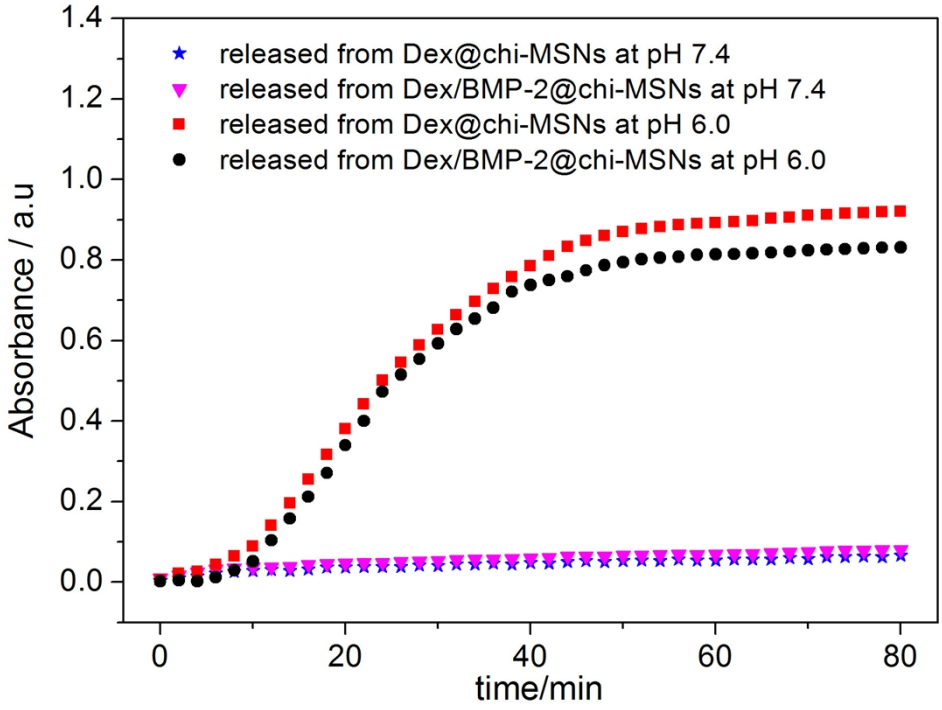

Fig. S6 In vitro release of dexamethasone from Dex@chi-MSNs and Dex/BMP-2@chi-MSNs in PBS at pH 6.0 and 7.4.

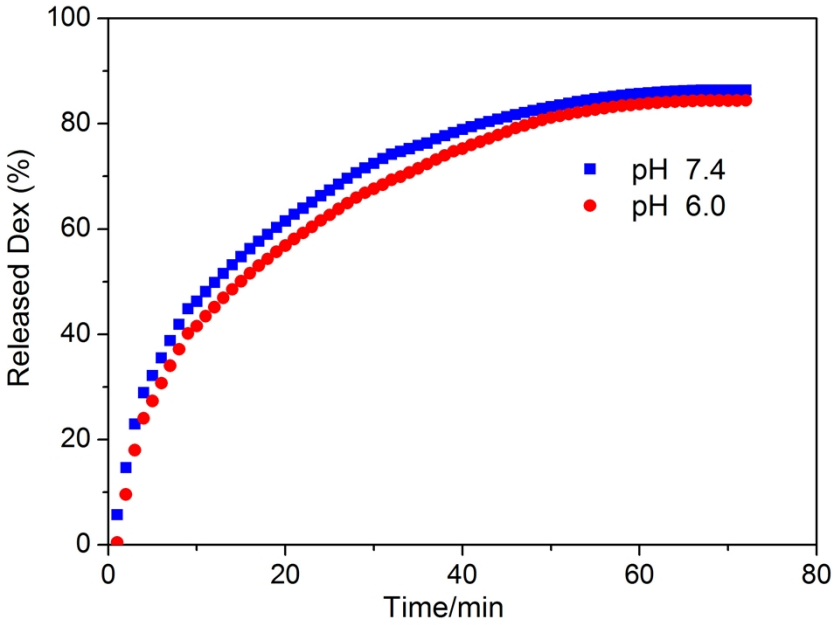

Fig. S7 Release profiles of dexamethasone in PBS from MSNs at pH 6.0 and 7.4.

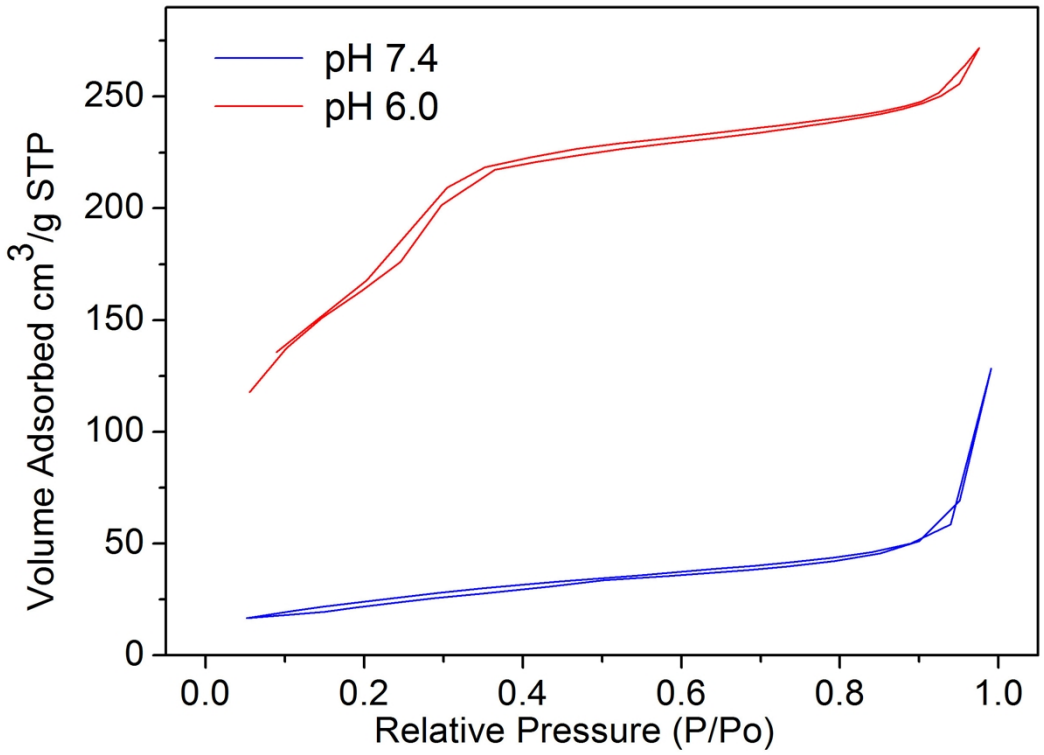

Fig. S8 BET nitrogen adsorption/desorption isotherms of chi-MSNs at pH 6.0 and 7.4. The chi-MSNs materials were immersed into PBS at pH 6.0 and 7.4 for 30 min, respectively. After that, the solution containing chi-MSNs was frozen for 8 hours and then freeze dried. The surface analysis of these materials was performed by nitrogen sorption isotherms in a Micromeritics ASAP2010 sorptometer.

Table S1. Sense and antisense primers utilized for real-time RT-PCR amplification.

Target	Forward primer sequence	Reverse primer sequence
ALP	5'-TATGTCTGGAACCGCACTGAAC-3'	5'-CACTAGCAAGAAGAAGCCTTTGG-3'
Runx2	5'-ATCCAGCCACCTTCACTTACACC-3'	5'-GGGACCATTGGGAACTGATAGG-3'
osteocalcin	5'- GCCCTGACTGCATTCTGCCTCT-3'	5'-TCACCACCTTACTGCCCTCCTG-3'
osteopontin	5'-CCAAGCGTGGAAACACACAGCC-3'	5'-GGCTTTGGAACTCGCCTGACTG-3'
Collagen I	5'-CTGCCCAGAAGAATATGTATCACC-3'	5'-GAAGCAAAGTTTCCTCCAAGACC-3'
β-actin	5'-CACCCGCGAGTACAACCTTC-3'	5'-CCCATACCCACCATCACACC-3'

Table S2. Secondary Structure of BMP-2 released from MSNs and chi-MSNs at pH 7.4/6.0 in PBS as determined by CD spectra [(a)].

Secondary structure compositions	Free BMP-2	BMP-2 from MSNs at pH 6.0	BMP-2 from MSNs at pH 7.4	BMP-2 from chi-MSNs at pH 6.0	BMP-2 from chi-MSNs at pH 7.4
α-helix (%)	12.4	8.6	8.5	15.4	15.7
β-sheets (%)	44.2	25.5	28.1	37.6	36.9
β-turns (%)	17.8	24.7	23.8	21.4	18.3
Rndm. Coil	23.6	43.8	42.0	26.4	28.1
Changes of the folding structure (%)	-	29.4	26.0	13.2	11.1

[(a)] CDNN V2.1 software was used to evaluate the secondary structure using the "complex" spectra category and the 190–260 nm region of the spectra.

Table S3. Surface properties of the chi-MSNs materials at pH 6.0 and 7.4 from nitrogen adsorption/desorption isotherms.

Sample	BET surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
chi-MSNs at pH 6.0	547.9	0.38	2.3
chi-MSNs at pH 7.4	152.1	0.15	-