Supplementary Information

Supplementary experimental methods

1. Protein adsorption and release profile of CMs

BSA was used as a protein model to study the adsorption amount and release profile of CMs. In order to determine the loading capacity of CMs, 1 mg of CMs were added into 1 mg/mL of BSA solution, and after incubating under continuous shake of 30 rpm at 37°C, the suspension was centrifuged and the supernatant fluid was collected for measurement of BSA concentration by a BCA protein assay kit, which was used for calculation of the protein loading amount on CMs.

To determine the release profiles of CMs, 50 μg of BSA was loaded onto CMs according to the loading method described in section 2.2.3. The BSA-loaded CMs were suspended in PBS at a ratio of 1 mg/mL under a continuous 30 rpm shake at 37°C. At predetermined intervals, a certain amount of supernatant was collected and released BSA concentration was measured by a BCA protein assay kit, followed by adding an equal volume of fresh PBS.

2. *In vivo* BMSC recruitment to a ectopic site

A model of ectopic implantation into thigh muscle pouches of mice, immunostaining and flow cytometry were adopted to measure the percentage of recruited BMSCs by different groups of scaffolds. Twelve male C57BL/6 mice (Silaike Inc. Shanghai, China) with an average weight of 25 g were used and randomly divided into 4 groups: MBG, B@M, (I+B)@M, I@CM+B@M. The prepared scaffolds were implanted into the muscle pouches of mice, then the overlying muscle and skin was sutured. 4 days after implantation, samples were harvested for BMSCs recruitment percentage evaluation via flow cytometry. In brief, PBS was thrust into the sample by a syringe to flush out the cells from different directions for several times until the tissue became transparent. Each suspension was divided equally to two group and both centrifuged to collect cell. The cells were washed and suspended with PBS again, sealed by anti-FcRII/III antibody for 30 min, followed by staining with monoclonal anti-

CD29, anti-CD44, and anti-CD45 antibodies (Abcam, Cambridge, UK) on ice for 30 min. The unstained group was set as blank control. Both group were analyzed on a flow cytometer (BD Accuri20 C6, USA) and by FLOWJO software (Tree Star, San Carlos, CA).

Results:

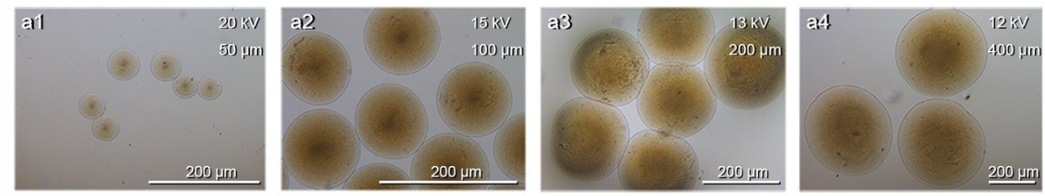

Fig. S1. (A) CMs of different diameters prepared under different voltages electrostatic fields and 4% TPP coagulation bath, observed by inverted light microscope (in solution): (a1) 20 kV, 50 µm; (a2) 15kV, 100 µm; (a3) 13 kV, 200 µm; (a4) 12 kV, 400 µm. Chitosan microspheres of diameters of 50, 100, 200 and 400 µm were synthesized under voltages of 20, 15, 13 and 12 kV, respectively, among which, the diameter of 100 µm formed under 15 kV voltage was selected considering the macroporous size of MBG scaffolds.

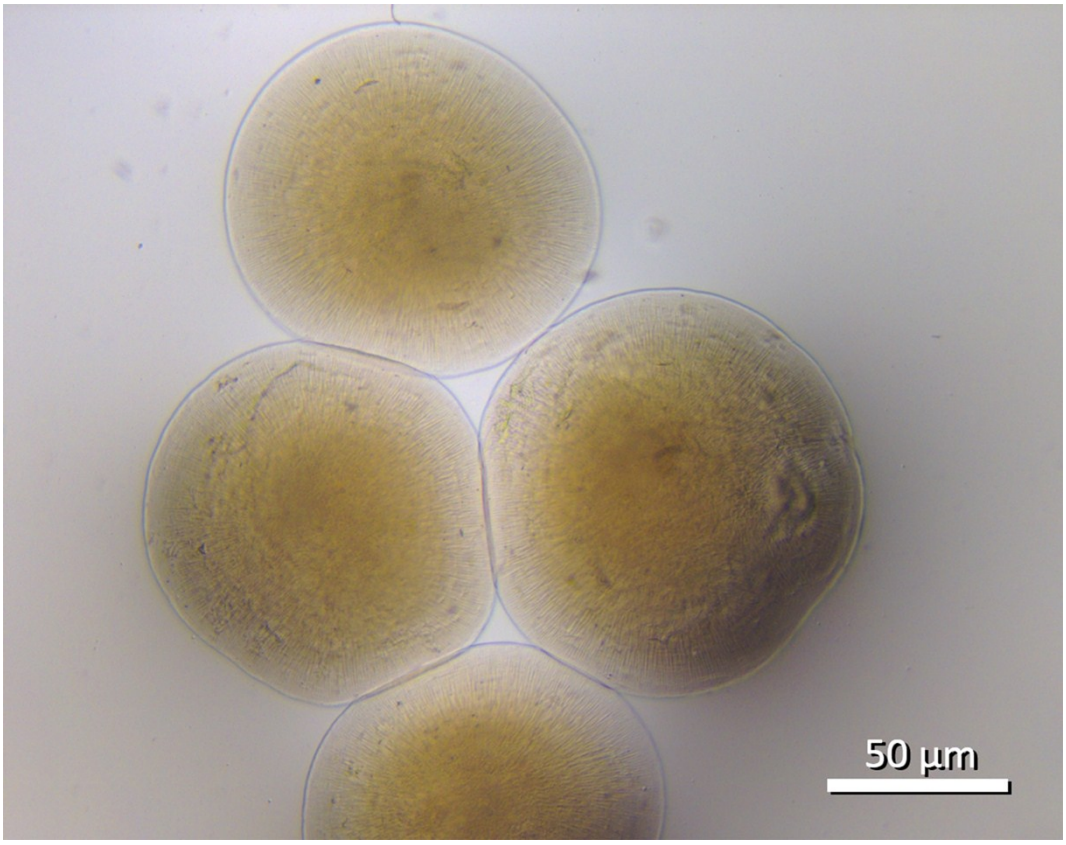

Fig. S2. Morphology of freeze-dried chitosan microspheres re-suspended in solution, observed by inverted light microscope (in solution). Though the microspheres were flattened after lyophilization, it would restore its sphericity in solution after absorption of water.

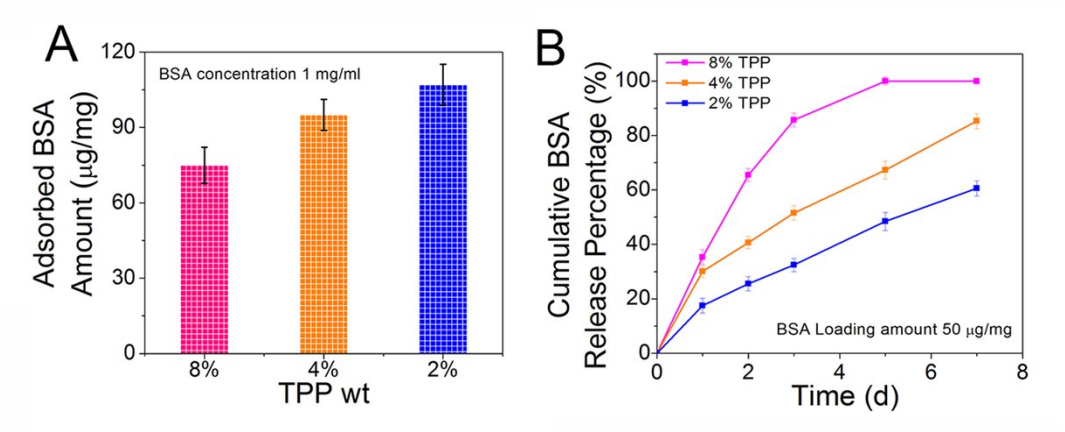

Fig. S3. Protein adsorption amount (A) and release profiles (B) of CMs of different crosslinking density, with BSA as a protein model. CMs of 100 μm diameter formed in 8%, 4% and 2% TPP exhibited BSA loading amount of 75, 95 and 107 μg/mg, and the 7-day protein release percentage from CMs formed in 8%, 4% and 2% TPP were 100%，85% and 60%, respectively. According to the function time of IL-8 in regenerative process, 4% TPP was chosen for CMs preparation.

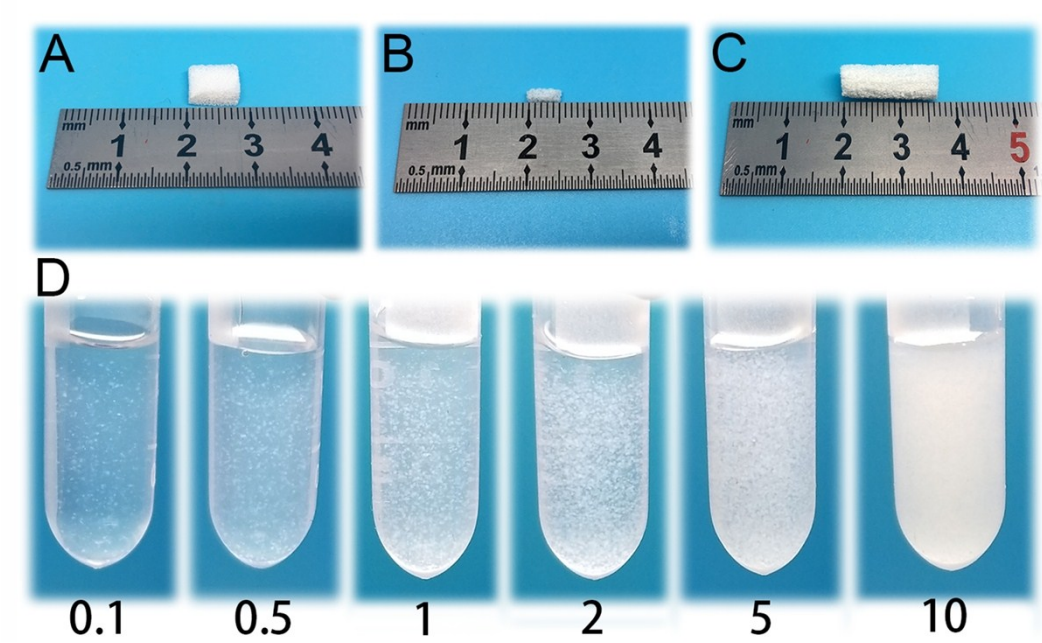

Fig. S4. Digital photographs of the materials in this study: MBG scaffold used in (A) cell experiments (8 * 8 * 3 mm^3); (B) BMSC recruitment evaluation in a model of ectopic implantation into thigh muscle pouches of mice (Ø 2mm * 5mm) (C) rabbit radius large segmental defect model (Ø 5mm * 16mm); (D) Gradient concentrations of chitosan microspheres in PBS (0.1, 0.5, 1, 2, 5, and 10 mg/mL).

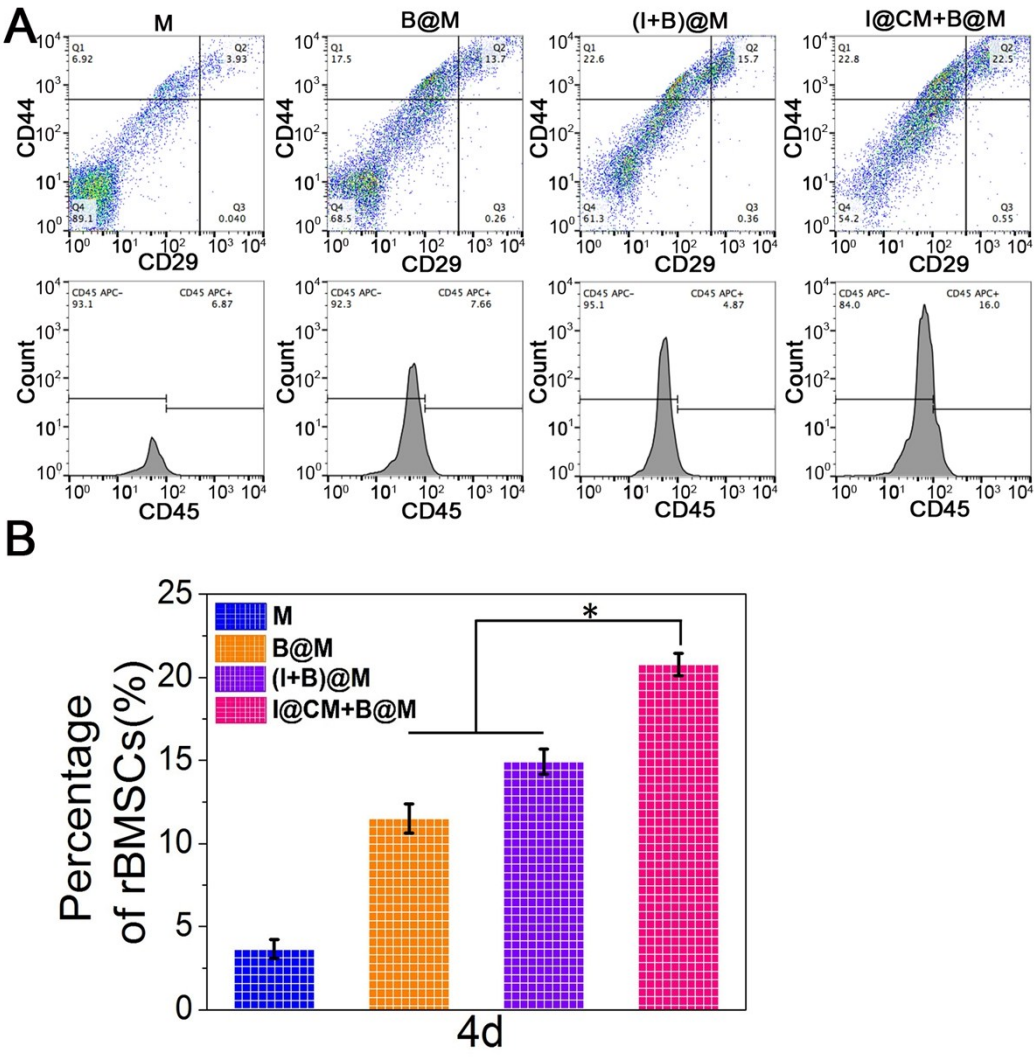

Fig. S5. *In vivo* recruitment of BMSCs to an ectopic implantation in thigh muscle pouches of mice by flow cytometry: (A) Flow cytometric profiles of sort gates of CD44-positive, CD29-positive and CD45-negative cells (criteria defining BMSCs); (B) Quantitative histogram of the percentage of recruited BMSCs. Asterisks indicate significant differences, $p < 0.05$. I@CM+B@M group exhibited the highest recruited BMSC percentage.

Table S1. Primers used in real-time PCR.

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Runx2	ATCCAGCCACCTTCACTTACACC	GGGACCATTGGGAACTGATAGG
Col 1	TGGATGGCTGCACGAGT	TTGGGATGGAGGGAGTTTA
OCN	GCCCTGACTGCATTCTGCCTCT	TCACCACCTTACTGCCCTCCTG
OPN	CCAAGCGTGGAAACACACAGCC	GGCTTTGGAACTCGCCTGACTG
β-actin	CACCCGCGAGTACAACCTTC	CCCATACCCACCATCACACC