Supplementary

Regenerating infected bone defect with osteocompatible microspheres possessing antibacterial activity

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Table S1 Primers designed for selected genes in relation to osteogenic differentiation of BMSCs, as well as for TNF and IL-6 genes to determine the in vivo anti-infection effect of M+D+Ag+B microspheres.

gene	forward primer (5′−3′)	reverse primer (5′−3′)
RUNX2	TCCAGACCAGCAGCACTCC	TCAGCGTCAACACCATCATTC
OPN	AATGAAGGGCCCTGAGC	GCCAGTTCTGCAAGGAAGC
COL-I	GCATGGCCAAGAAGACATCC	CCTCGGGTTTGGACGTCTC
OCN	AACGGTGGTGCCATAGATGC	AGGACCCTCTCTCTGCTCAC
TNF	GGCCAATGGCATGGATCTCA	ATGGCAAATCGGCTGACGG
Il-6	ACTTCCAGCCAGTTGCCTTCT	GGTCTGTTGTGGGTGGTATCCT
18S	AATGAAGGGCCCTGAGC	GCCAGTTCTGCAAGGAAGC

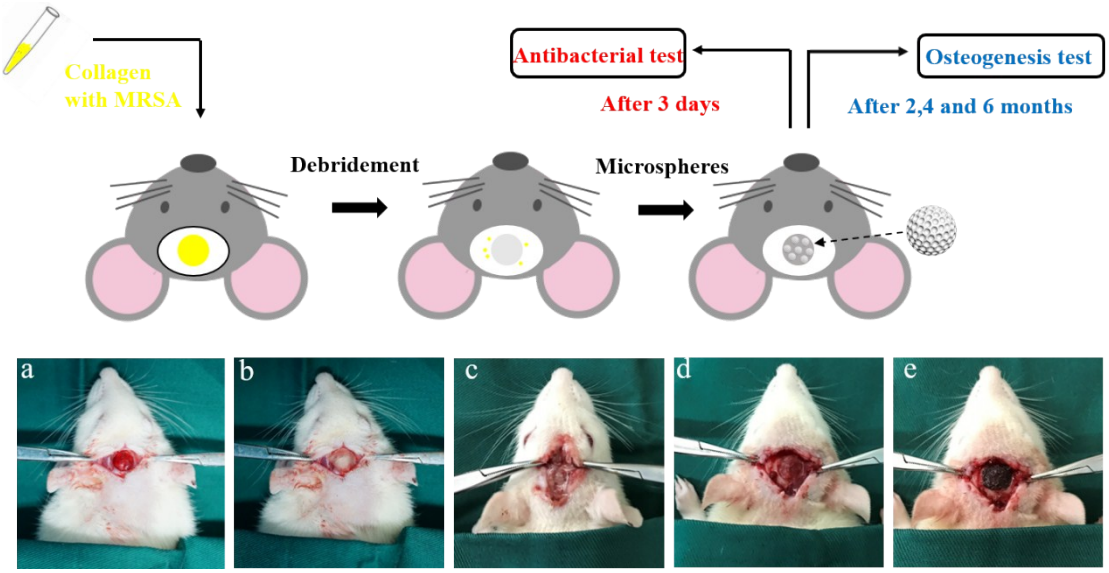

Fig. S1 Schematic process of generating infected bone defects. (a) The critical-sized circular defect was created by means of 8 mm-diameter trephine burr on the central region of the calvarium. (b) A resorbable collagen sponge pre-soaked with of S. aureus suspension (10^7 CFU in 100 µL of sterile normal saline) was inserted into the defect. (c) Obvious infection was clinically observed after one week. (d) Debridement was performed and infectious tissue was removed. (e) Microspheres were implanted into the infected defects, and the incisions were closed again.

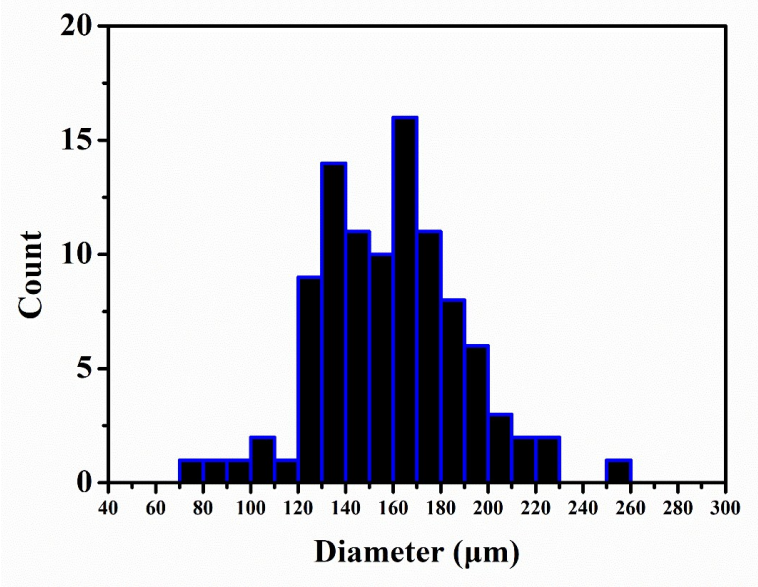

Fig. S2 The statistical analysis on size distribution of M+D+Ag+B microspheres basing on multiple SEM images with 100 microspheres being measured.

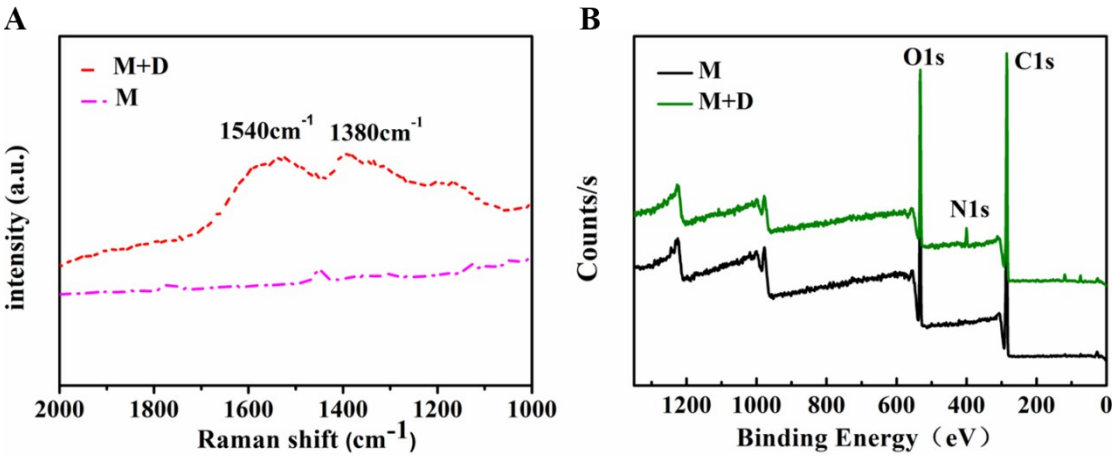

Fig. S3 Comparison between (A) Raman spectra and (B) XPS profiles of prepared M and M+D microspheres.

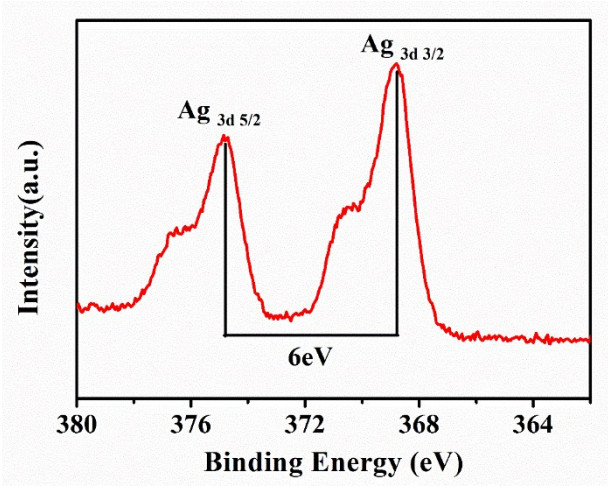

Fig. S4 The enlarged Ag_{3d} signal composed of two individual peaks at 368 eV and 374 eV being shown.

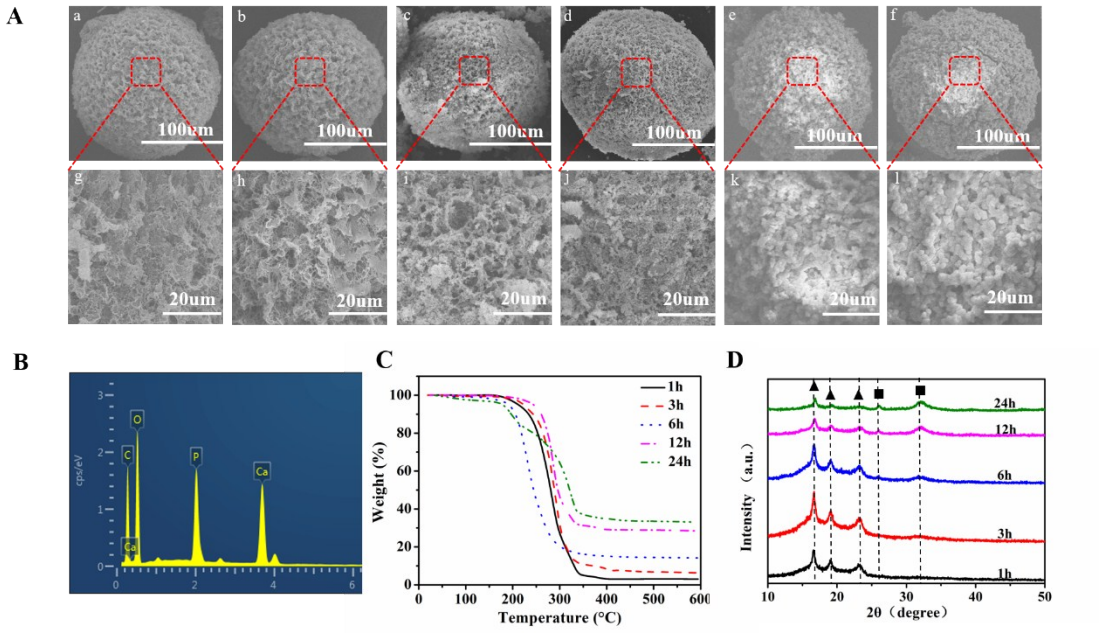

Fig. S5 (A) SEM micrographs of microspheres biomineralized for 0 h (a, g), 1 h (b, h), 3 h (c, i), 6 h (d, j), 12 h (e, k) and 24 h (f, l). (B) EDS of microspheres biomineralized for 12 h. (C) TGA curves and (D) XRD patterns of microspheres biomineralized for different times. In (D), ▲ indicates peaks assigned to PLLA-PEG-PLLA copolymer and ■ indicates signals assigned to HA.

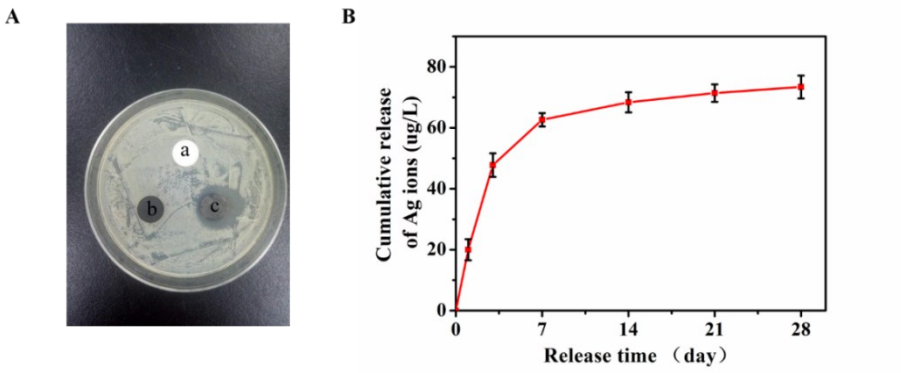

Fig. S6 (A) antibacterial activity to S.aereus of M microspheres (a), M+D+B microspheres (b) and M+D+Ag+B microspheres, together with (B) the release behavior of silver ion from the M+D+Ag+B microspheres.

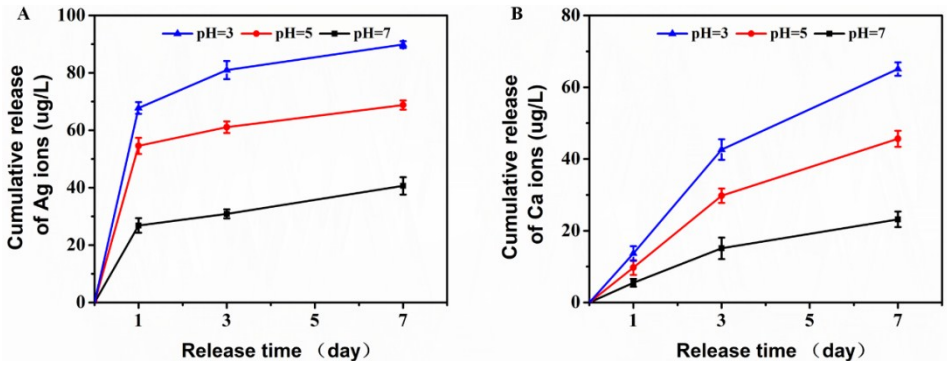

Fig. S7 The release behaviors of (A) silver and (B) calcium ions from M+D+Ag+B microspheres under different pH values at 37°C.

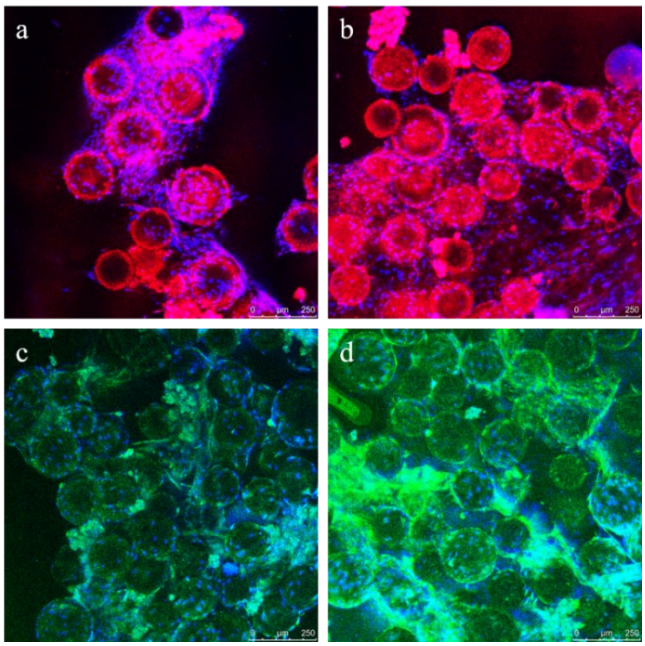

Fig. S8 Immunofluorescent staining to show the abundant expressions of OPN (a, b) and OCN (c, d) for BMSCs cultured on M+D+B (a, c) and M+D+Ag+B (b, d) microspheres for 14 days.

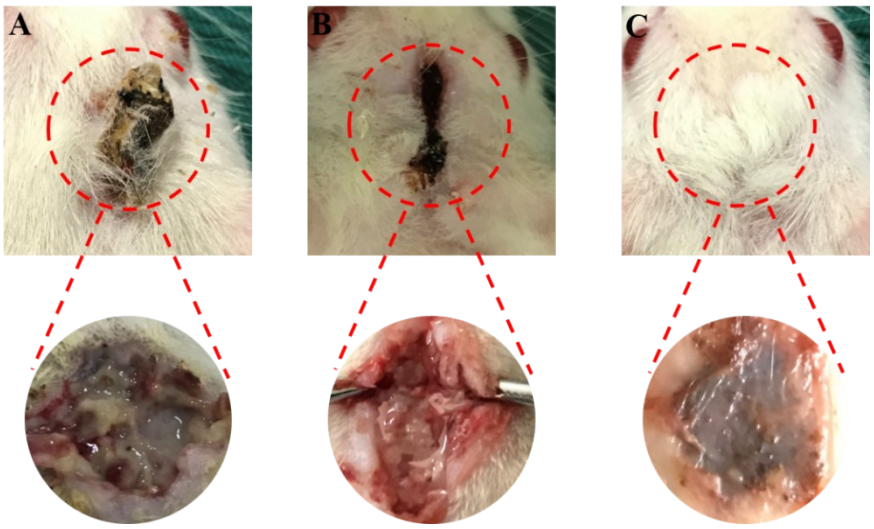

Fig. S9 Macroscopic observations to show the infected cranial defect areas of SD rats one month post-operation in control group (A), M+D+B microsphere-filled group (B) and M+D+Ag+B microsphere-filled group (C).

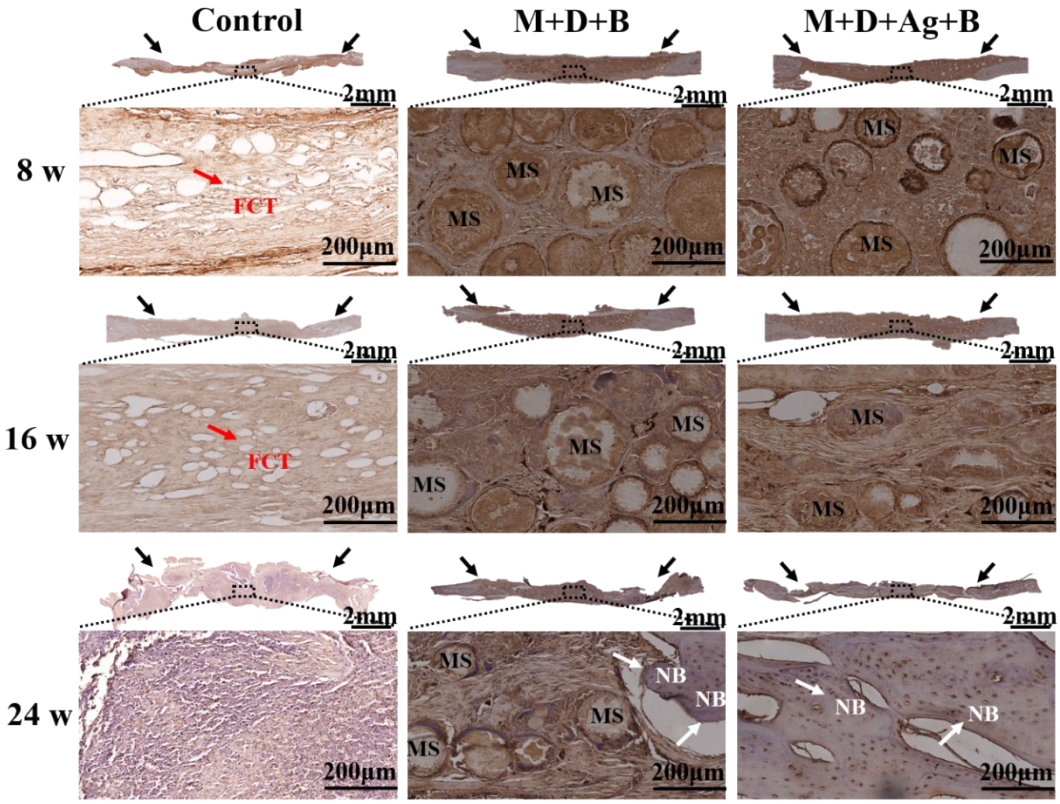

Fig. S10 Immumohistochemical staining on OPN expression to evaluate the regeneration of infected bone defects at 8, 16 and 24 weeks post-operation for the control group and groups filled with M+D+B or M+D+Ag+B microspheres. Red arrows indicate the fibrous connective tissue (FCT), MS represents the location of residual microspheres, and white arrows indicate the newly formed bone (NB).

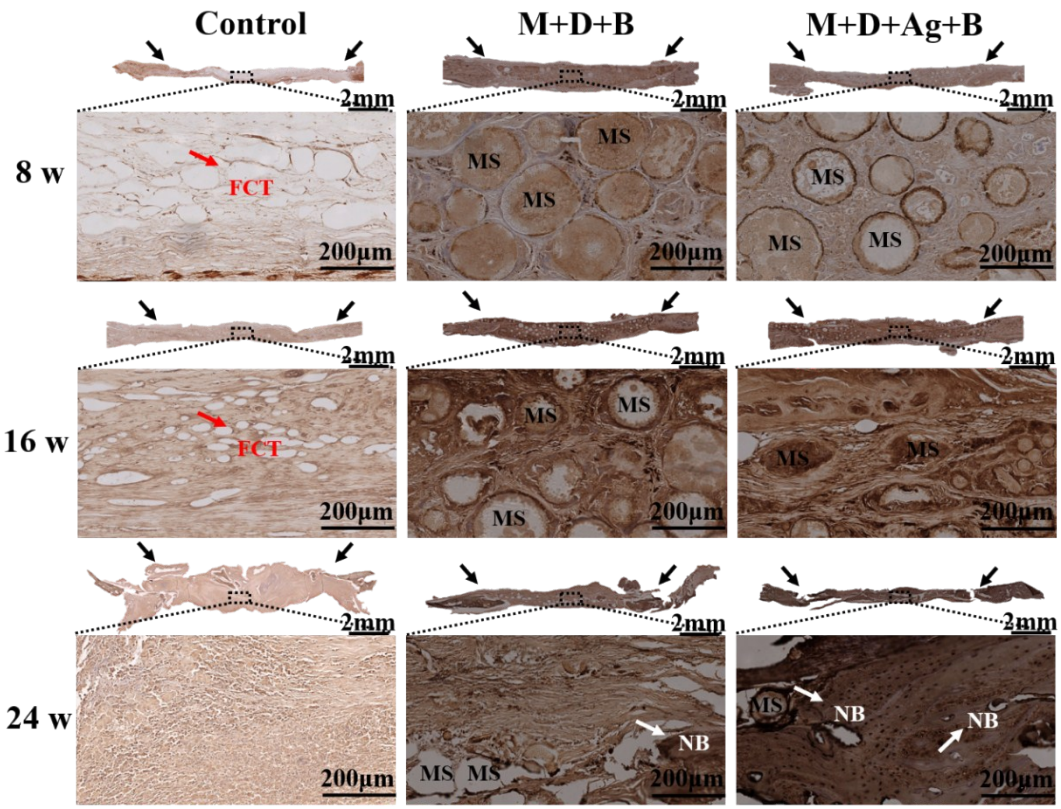

Fig. S11 Immumohistochemical staining on OCN expression to evaluate the regeneration of infected bone defects at 8, 16 and 24 weeks post-operation for the control group and groups filled with M+D+B or M+D+Ag+B microspheres. Red arrows indicate the fibrous connective tissue (FCT), MS represents the location of residual microspheres, and white arrows indicate the newly formed bone (NB).